INFLUENCE OF FERTILITY LEVELS ON SUGAR CONTENT AND COLDHARDINESS IN COMMON BERMUDAGRASS (*Cynodon dactylon* (L.) Pers) RHIZOMES, AND IDENTIFICATION OF SOME SUGARS

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ABBREVIATIONS

Arab	Arabinose
D. F.	Degrees of Freedom
Elec.	Electrolysis
Fre	Freeze
Fru	Fructose
Gal	Galactose
Gal A	Galacturonic Acid
Glu	Glucose
K	Potassium
M	Month
mu	Millimicron
Malt	Maltose
Man	Mannose
Mo	Month
N	Nitrogen
Nit	Nitrogen
No	Node
Nod	Node
P	Phosphorus
Phos	Phosphorus
Pot	Potassium
Read.	Reading
Rep	Replication
Suc	Sucrose
ug	Microgram
Unk	Unknown
v/v	Volume per volume

CHAPTER I

INTRODUCTION

Due to its growth habit and ability to grow on many different types of soils, common bermudagrass (Cynodon dactylon (L.) Pers) is widely used as an erosion control grass on roadbanks, pond dams, waterways and other areas requiring stabilization.

Winterkill of bermudagrass is not uncommon in Oklahoma. From six years' experience with the Soil Conservation Service, the author found that newly planted areas were particularly susceptible to winterkill the first winter after establishment. The cost of establishing a bermudagrass sod on one mile of highway roadbank can exceed $50,000.00. The re-establishment of winterkilled areas on roadbanks and adjacent areas is a major problem of the Oklahoma Department of Highways.

The purpose of this study is to: (a) find a quick and simple method of determining sugar content of bermudagrass rhizomes; (b) determine the effects of various rates of fertilizer on the sugar content of the rhizomes; (c) determine if sugar content in the rhizomes is correlated with winterhardiness; and (d) determine which sugars are present in common bermudagrass rhizomes.

If fertilizers can increase coldhardiness in bermudagrass, this would be a simple, and relatively inexpensive, method of reducing winterkill on newly planted areas; and could result in substantial savings for the Oklahoma Department of Highways.

CHAPTER II

LITERATURE REVIEW

Bermudagrass is grown on millions of acres in the United States (41). It is one of the major forage grasses in the south and, due to its growth habit, is also widely used for erosion control purposes.

Much of this area is in the deep south where winterkill of bermudagrass generally is not a problem. Consequently, only a limited amount of work on winterhardiness of bermudagrass has been done. Most of this research has been oriented primarily toward determining the effects of fertilizer on winterhardiness in bermuda (1, 21, 77). Davis and Gilbert (12) have investigated the soluble proteins in bermudagrass in an attempt to determine changes in this fraction during cold hardening, and Ahring and Irving (3) concentrated on a laboratory method of determining coldhardiness.

Winter injury of bermudagrass in Oklahoma has been seen many times by the author. Winter injury of grasses may be the result of one particular condition occurring at one particular period during the winter, or it may be the result of several conditions occurring at one period, or during successive periods of the winter (95).

The two major components of coldhardiness seem to be (1) avoidance of intracellular freezing, and (2) tolerance of intercellular freezing (48). However, there is a considerable difference of opinion as to the mechanisms involved during these processes which actually result in

damage to the cells.

Effects of Ice Crystal Formation

In nature, temperatures normally drop relatively slowly. When this occurs, ice crystals first form in the plant in areas where the water contains the lowest concentration of solutes, the xylem and the intercellular spaces. The formation of ice crystals in these areas causes a vapor pressure deficit which results in water being moved from inside the cell to join the ice crystal outside the cell. This movement of water out of the cell results in several physical alterations; (1) a concentration of solutes, (2) precipitation of solutes, (3) reduction in cell water content, (4) cell shrinkage or plasmolysis, (5) changes in pH, and (6) a reduction in the spatial separation of macromolecules (57).

Several of these changes have been suggested as the primary cause of freeze injury by various individuals. Meryman (61) proposes that the cell membrane is injured by the osmotic pressure gradient being greater than that compensated for by cell volume change. He believes the damage is unrelated to the concentration or identity of the intracellular solvent, but is directly related to the proportion of the cell liquid phase removed (62). This theory is supported by Heber (26), who believes the basic cause of injury is the alteration of permeability properties of the membranes due to the dehydration accompanying freezing. Mazur (57), on the other hand, believes there is a direct interaction of ice crystals with the membrane system which causes the damage rather than the indirect effects associated with the loss of liquid water. Lovelock (52) thinks that the principal damaging effect

associated with freezing is due to the concentration of both intra and intercellular electrolytes.

Sakai (85), who worked with twigs of woody plants, froze them slowly and then immersed them in liquid nitrogen at -196° C for as long as 160 days with 100% survival. He concluded that there was a definite temperature at which almost all of the easily freezable water in a cell may be drawn from the cell interior by extracellular freezing. Cells and tissues in this state are not injured even when exposed to extremely low temperatures. Thus, the low temperature itself could not be the primary factor in freeze damage.

The work by Sakai gives support to those who believe that the water remaining after the initial freezing period provides protection from freezing injury. This water is normally termed "bound water" and definitions vary with the researcher. Meryman (63) lists 14 different definititions. Levitt (43) found that hardened cabbage (Brassica L.) leaves retained larger amount of bound water than did unhardened leaves. According to Olien (71), most of the extracellular water closely associated with the cells in wheat leaves exists as a film surrounding the living protoplasts. According to Meryman (63), Hori, worked with films of water between two glass plates, and found a steadily decreasing temperature of spontaneous freezing as the film thickness was reduced from one to .01 mm, at which point the water froze at about -30° C. Thinner films did not freeze even at -100° C.

Olien (72) concluded that the structure of ice crystals forming between the protoplasts is the most important factor affecting initial injury. Masses of small or imperfect crystals cause little damage. These masses become more perfect, solid, and destructive as the

temperature drops and vapor from protoplasts adds to their size.

When the temperature drops rapidly, or drops low enough during a slow freeze, intracellular ice formation always results in death of the cell. This was mistakenly believed to be due to the ice formation causing cell rupture (71). However, since the development of the electron microscope, it has been found that intracellular ice formation does not always result in death of the cell.

Sakai and his associates (84, 86, 87) showed that rapid freezing to extremely low temperatures resulted in small imperfect ice crystals being formed within the cells. If the cells were thawed rapidly, there was no damage. However, when rewarming took place slowly, the ice crystals gradually grew larger through a recrystallization process, even at temperatures much lower than freezing. They eventually reached such a size that the viability of the cells was destroyed. Although the masses of ice were occasionally large enough to cause rupture of the cells, usually the cell microbodies were damaged, with a resultant disruption in the permeability properties of the cell membrane. Mazur and Schmidt (59) also provided data which supported the work of Sakai and his group.

Effect of Solutes Within the Cell

Due to the increase in concentration of the intracellular materials, many studies have been done on the various solutes found in the cell. Although some contested the theory (29), Chandler (10) was one of the earliest to recognize that the ability of plants to withstand low temperatures increased as the sap density of the tissues increased. In recent work, Mazur (58) explained the response of microorganisms to

freezing in terms of solute concentration and intercellular freezing. Levitt (47) increased frost hardiness using glycerine solutions and thought that the osmotic effects of the solute could completely and quantitatively explain the increase. Heber (27) found a number of neutral solutes capable of providing protection. A prerequisite appeared to be the ability to form H+ bonds. Sugars such as arabinose, glucose, and sucrose were found to be highly protective. With increasing molecular weight, the effectiveness decreased until higher molecular weight compounds such as dextrans and soluble starch were no longer protective.

Meryman (61) felt that the criteria for a freeze protective agent were merely that it be nontoxic in high concentrations and would penetrate the cell freely. Lovelock (53) verified this idea when, working with human blood cells, he showed that glucose and xylose, to which the cell is only partially permeable, offered only partial protection; and sucrose, to which the cell is impermeable, offered little or no protection. Heber (26) also found that freezing altered the permeability of chloroplast membranes and that they no longer functioned as osmometers when frozen. He assumed that freezing uncoupled phosphorylation from electron transport. He found that several energy requiring activities could be protected against inactivation by the addition of sucrose prior to freezing.

Hardening in Plants

The actual development of resistance to freezing injury in plants, or "hardening," is so closely related to freezing damage that it is often studied at the same time. Some plants are fairly stable and their

hardiness fails to change much with external conditions. Yet in spite of these exceptions, the autumn rise in frost tolerance is a normal phenomenon found in the hundreds of species and varieties tested by investigators throughout the world (48).

As early as 1898, Lidforss (51) found that all of the starch was being converted to sugar during the cold season of the year in evergreen plants. At the same time he found underwater plants, not subject to freezing, remained filled with starch; and they also did not acquire frost resistance. Rosa (81) found substantially the same changes in different plants. Since these early workers, many others have found the same starch to sugar conversions during hardening in many other plants (7, 34, 37, 50, 56, 75, 92, 108).

A very precise temperature control mechanism sensitive to small temperature changes was found by Marvin and Morselli (55). A single degree centigrade induced a rapid hydrolysis of starch in maple (Acer saccharum Marsh.) stems. Some workers however, did not find the uniform change in sugar as cold increased (104), or found that the sugar content did not vary uniformly with winterhardiness (68).

Many other factors have been shown to influence hardening. Harvey (25) induced greater hardening in cabbage by a one-hour exposure at 0° C than he did with a longer exposure at a higher temperature, and suggested that hardening is a cold shock response. Dexter (14) showed that low CO_2 levels prevented hardening, and he and several others showed that short day length increased hardiness (31, 38, 75).

Steponkus and Lanphear (101), admitting that light greatly enhanced the degree of hardiness in English ivy (Hedra helix L.), thought that it was not obligatorily linked to hardiness. They believed that the

light requirement varied from species to species and that it might work as a phytochrome response. One explanation of the influence of day length on plants was given by Irving and Lanphear (32) when they showed that gibberellin acted as an inhibitor to cold hardening. Moblidowska (65) found the same effects of gibberellic acid; however, his results were somewhat inconsistent. Kohn and Levitt (38) thought there was another factor related directly to low temperature rather than light exposure.

Proteins and Hardening

Working with English ivy, Parker (75) found a gradual increase in water soluble proteins as hardening occurred, as well as an increase in sugars and anthocyanins. In the spring the sugars and anthocyanins decreased along with the winterhardiness. Other workers have found an increase in proteins related to increasing hardiness (11, 20, 24, 27, 34, 35, 36, 48, 50, 88, 90, 91, 110). Jung and Smith (34) determined that alfalfa (Medicago sativa L.) was more winterhardy than red clover (Trifolium pratense L.), and then found that water soluble protein nitrogen was the only fraction consistently higher in alfalfa than in clover. Li and his associates (50) also found an increase of protein in Red Osier dogwood (Cornus stolonifera Michx.) as hardiness increased. They found a decrease in amino acids while Pauli and Mitchell (76) found free amino acids and amides were higher in hardened wheat plants.

Three major changes during hardening were found by Davis and Gilbert (12), who investigated the soluble protein fractions of bermudagrass with polyacrylamide gel electrophoresis. The changes were (1) an increase in total protein concentration, (2) a decrease in density of

two closely spaced bands of protein, and (3) an increase in the total number of protein bands. Four of the bands appeared between the 15th and 30th day, indicating that bermudagrass hardens over an extended period. Gerloff, Stahmann, and Smith (20) extracted water soluble proteins from hardened and non-hardened alfalfa roots. Soluble proteins increased during hardening in all varieties and two new iso-enzymes with peroxidase activity were found in the fully hardened samples. Peroxidase and catalase activity increased during hardening in all varieties; however, they found only small differences between hardy and non-hardy varieties. On the other hand, Hall and his associates (24), who studied enzymes in willow (Salix nigra Marsh) stems, found the total protein banding pattern was unaltered while freezing tolerance changed 70° C over a three-day period. They also found no change in peroxidase banding. They thought the possibility of one or a few proteins providing protection was unlikely.

Heber (27) corroborated the work of Davis and Gilbert, and Gerlof's group, by isolating two small proteins from hardy spinach (Atriplex oleracea L.), rye (Secale L.) and Valerianella (Mill.). These two fractions, called Proteins I and II, on a unit weight basis, were from 20-50 times as effective as sucrose in preventing the inactivation of photophosphorylation due to the freezing of chloroplast membranes. On a molar basis, the factors were 2500 times more effective than sucrose. Heber felt the existence of these highly active protein factors might explain why hardiness is closely related to sugar content only in some plant species and not in others. With high winterhardiness and low sugar (pines), the protein fractions may be the protective agent. Siminovitch and Brigg's (91) research with black locust (Robinia

pseudoacacia L.) led them to believe that some factor, or factors, must reach the cells before the water soluble proteins can be synthesized. This factor appeared to be mobilized from the leaves only in late summer and autumn, reaching maximal transport in September just prior to leaf abscission.

Several specific proteins have been identified as being associated with hardening. Jung, Shih and Shelton (36) found DNA and RNA increased during hardening in alfalfa. A system of protein protection based on attachment of sulfhydrl (SH) groups to the protein molecules was proposed by Levitt (49). He used cabbage cells and confirmed Maximov's theory on the effect of non-penetrating, nontoxic solutions against freezing. However, he could find no evidence of freezing protection by surface sulfhydrl groups. He did conclude that since the protection is connected with plasmolysis during freezing, it cannot be explained by a surface effect but there must be protection for the internal cytoplasmic proteins. In later work, he found that SH reagents increased freezing injury. This, of course, was in opposition to his original theory. He did show that they induced some noninjurious protein unfolding which favored the aggregation of proteins on freezing (45). Heber and Santarius (28) used cysteine and glutathione in an attempt to protect the chloroplast system of spinach based on Levitt's SH theory. They could detect no oxidation of protein SH groups due to freezing and consequently rejected the SH theory.

Working with black locust, Siminovitch and his group (92) suggested that normal hardening is a composite of three principal processes which include membrane replication, membrane replication in association with whole protoplasmic augmentation and starch to sugar conversion. Li,

Weiser, and Van Huystee (50), in their work with dogwood, also found a decrease in inorganic phosphorus as hardiness increased and an increase in bound phosphorus and total organic phosphorus.

An explanation of the phosphorus changes was proposed by Stewart and Guinn (102). They found higher levels of ATP in hardened cotton (Gossypium L.) seedlings than in unhardened plants. An explanation for the lack of membrane integrity found by many workers was also provided. At low temperatures the mitochrondia became less functional and the ATP in the system was used up. Without re-synthesis of ATP, a general decrease in phosphorylated compounds would result. Below a certain point of available energy, the tissue could no longer maintain the metabolic integrity of the cytoplasm necessary for survival. Structural damage could not be repaired and general disorganization of cellular structure and metabolic processes would occur with depletion of the usable cellular energy. This theory would explain the membrane changes listed by many workers.

Kuraishi et al. (40), who worked with pea (Lathyrus L.) plants, found that hardened plants had low NADP and high NADPH levels while unhardened plants had just the reverse concentration. Heber and Santarius (28) also found that freezing damaged ATP synthesis through an uncoupling effect.

Jung, Shih and Shelton (35) found a significant reduction in winter injury of the nonhardy alfalfa variety, Caliverde, occurred with the application of guanine, adenine, and cytosine. The amino acids were applied in the fall as a foliar spray. Injury to the hardy variety, Vernal, was significantly reduced by the application of guanine, thymine and cytosine. Sometimes the chemical treatments induced

coldhardiness in Caliverde equivalent to that of the untreated Vernal. Jung suggested that coldhardiness resulted from a complex genetic-chemical-treatment-environment interaction.

The same alfalfa varieties were used by Nath and Fisher (67) in a similar study. They used only cytosine and guanine and found that while the cytosine treatment appeared to reduce histological freeze damage in both varieties, guanine had little or no effect. Shih and Jung (90), in a more recent study with Vernal and Arizona Common alfalfa, found that guanine and cytosine altered metabolism which resulted in a higher concentration of water soluble protein.

Sugars and Hardening

In the same study, Shih and Jung (90) also found that amino acids had no effect on the concentration of soluble sugars. It was concluded that the reducing sugars, which are only a small part of the total sugars, are not associated with winterhardiness in alfalfa. These researchers found that sucrose was the sugar present in largest amounts, and it was positively correlated with winterhardiness in alfalfa.

Heber and Santarius (28) found that the uncoupling effect of freezing on ATP synthesis could be prevented by the addition of small amounts of glucose, sucrose and raffinose to the chloroplast system. It was found that oxidative phosphorylation of mitochrondia could be preserved by sugars. Thus, they decided that sugars protect the cells by retaining or substituting water via hydrogen bonding in structures sensitive to dehydration. During freezing, the hydrogen binds the hydroxyls of the sugar to functional water of the membrane system, or directly to sensitive sites of that system. This results in protection, since

sugars, contrary to water, are not frozen out under conditions prevailing in the cell, and rupture of the stabilizing bonds is no longer possible.

A large number of reports has accumulated over the years concerning the protective action of sugars, and the amount of sugar present in hardy and non-hardy plants (4, 23, 64, 69, 82, 88, 96, 100, 105, 110). As early as 1927, Akerman (4) studied the sugars present in ten varieties of wheat. The varieties varied from extremely hardy to extremely susceptible. In every case the sugar content was directly correlated with the winterhardiness of the wheat variety. The same results were reported by Richter (78) who worked with rye, wheat, and rye-wheat hybrids. The sugar content was highest in the most hardy, and lowest in the least hardy.

According to Okajima and Smith (70), De Cugnac showed that two groups of perennial grasses could be distinguished according to the type of reserve carbohydrate stored in the overwintering vegetative parts. One group was characterized by the accumulation of fructosan together with sucrose. The second group did not contain fructosan, but stored sucrose and starch. The starch containing grasses appeared to be native to the semitropical or tropical latitudes, while fructosan containing grasses appeared to be native to the temperate latitudes. Several other workers found the same results working with these two groups of grasses (22, 94, 96, 97).

Starch, sugars, protein, peroxidase activity, RNA, DNA, fatty acid changes, and mineral changes of nitrogen, phosphorus, potassium, calcium and magnesium were studied by Smith (96). He found that only the overwinter trends of total free sugars and water soluble protein

nitrogen were consistently correlated with the overwinter trends of frost hardiness. Sakai and Yoshida (88) found that sugar and sugar alcohol were the most effective among 60 compounds used in preventing freeze injury of cabbage leaves in slow freezing and thawing. They also found that non-hardy cells didn't harden, but sugar levels increased, depending on the species, and the stage of development of some species. They also considered the conformational change in membrane systems as the primary factor controlling seasonal variations in freezing resistance.

Frost-hardened cabbage leaves were infiltrated with dextrose or fructose by Levitt (44). He obtained an increase in hardiness which could be calculated prior to testing based strictly on the osmotic effects. McKell, et al. (60) grew Coastal bermudagrass (Cynodon dactylon (L.) Pers. var. Coastal) under controlled conditions to check the effect of various temperature regimes. They found that the lowest temperature regime showed the largest accumulation of starch and no difference in the soluble sugars. However, since their lowest temperature was 6.7° C, it is possible that the temperature was too warm for a significant hardening effect to occur.

Cortical cells of mulberry (Morus L.) were treated by Sakai and Otsuka (86) with 2 molar solution of sugars. The cells were then hardened for 10 days at 0° C, and were found to resist freezing at -20° C. Without the sugars, the cells survived only -5° C. They believed that the basic factor was not whether ice was formed within the cells, but rather the size of the ice crystals. Sakai used various sugars to evaluate the amount of protection they provided to cabbage cells. He listed them in groups which provided equal protection. In the order of

decreasing protection, the groups were rhamnose and xylose; glucose, fructose and galactose; sucrose and sorbital; lactose; raffinose and mannitol. A similar study was done with winter wheat by Tumanov and Trunova (105). They reported that sucrose provided the most protection followed closely by glucose. Then, in the order of the decreasing amount of protection provided, came rhamnose, lactose, maltose and galactose. It was found that these sugars were taken up by the plant and converted into different sugars in the cells. The new sugars provided the actual protection. Tillering nodes and leaves were able to make this conversion, coleoptiles were not. Winterhardiness was based on the enzymes being present in the cells to form the new sugars.

Sakai (83) found that the sugar concentration in the cortical cells contributed to the decrease of the growth rate of the crystallites which are formed in the course of rapid cooling. Olien (73) extracted water soluble polysaccharides from leaves of a hardy rye variety and a less hardy barley variety and found that the ice structure in the frozen solutions of the rye polymers was much less perfect than in solutions of the less hardy barley. A study of the kinetics showed that the effect was not on the freezing point or on the organization of liquid water. The polymers interacted strongly with the ice-liquid interface and interfered with freezing. In a later study, he found that the polymers, which affected the kinetics of freezing, interfered with freezing by competing with water for positions in the ice lattice, rather than competing with ice for liquid water as do most anti-freeze compounds. These polymers acted as competitive inhibitors of a catalyzed reaction, with the ice-liquid interface as the catalyst. The interference did not prevent freezing, but greatly upset the structure of the ice (74).

This work could provide an explanation for the greater coldhardiness of cool-season grasses as opposed to the warm-season grasses. The high concentration of fructosans results in the type of protection disclosed by Olien's work. Lacking the large concentration of polymers, the warm-season grasses would be much more susceptible to freeze damage.

Soil Fertility and Coldhardiness

As the development and use of fertilizers has increased, so did the research into the effect of fertilizers on coldhardiness. Dexter (16) found that quackgrass (Agroypron repens L.) fertilized with nitrogen failed to harden while the unfertilized grass did harden. Carroll and Welton (9) found that Kentucky bluegrass (Poa pratensis L.) plots receiving high nitrogen rates were less resistant to cold than plots receiving low nitrogen rates. The plants grown under high rates of fertilizer also contained lower amounts of sugars and pentosans than those grown under lower rates of fertilizer. Carroll (8) showed that the amount of sugars and bound water were decreased by nitrogen fertilization of turf grasses. Adegbola and McKell (2) studied Coastal bermudagrass fertilized with various rates of nitrogen. They checked reducing sugars (glucose and fructose) and non-reducing sugars (sucrose) in samples taken every two weeks for a full growing season. Although there was an increase in reducing sugars in the leaves with each added increment of nitrogen, there was no change in the sugar content of the rhizomes, but sucrose and fructosans in the rhizomes decreased with increasing nitrogen fertilizer rates. Adams and Twersky (1) found that survival of Coastal bermudagrass increased with increasing levels of potassium fertilization at each nitrogen level. At any potassium level,

survival decreased with an increase in nitrogen fertilizer. They failed to report the level of phosphorus fertilization used in this study.

MacLeod (54) studied nitrogen-potassium fertilization of Cayuga alfalfa (Medicago sativa L. var. Cayuga), smooth bromegrass (Bromus inermis Leyss. var. Saratoga), orchardgrass (Dactylis glomerata L. var. Froede) and timothy (Phleum pratense L. var. Climax). Significant interactions were found between the grass species and phosphorus and potassium. These results indicated that potassium fertilization facilitated the storage of carbohydrate reserves at high rates of nitrogen, and that high rates of potassium without nitrogen fertilization were detrimental to the storage of carbohydrate reserves.

Howell and Jung (30) checked the levels of potassium, sodium, and calcium in orchardgrass sap. They found the levels of all three elements were, in general, directly proportional to the level of cold resistance of the plants; and that the concentration of potassium was much higher than that of sodium or calcium. They reported that neither stage of growth nor nitrogen fertilization altered potassium or calcium content of the sap, although sodium levels were affected by both.

Fourteen hardened cool-season turf species were checked for hardiness by Beard (6). He decided two of the most important cultural factors leading to reduced coldhardiness were excessive nitrogen and a deficiency of potassium. Wang, Attoe and Truog (108), who worked with alfalfa, found that the addition of lime, lime plus phosphorus, or lime plus potassium, or lime plus phosphorus plus potassium, all gave increased non-reducing sugars but no change in reducing sugars. The addition of phosphorus plus lime, and phosphorus plus lime plus potassium also increased starch. Winterhardiness was increased by large amounts

of both starch and non-reducing sugar in the plant tissue. The addition of all three minerals resulted in increased water soluble protein which also increased coldhardiness. They concluded that high levels of lime and available phosphorus and potassium (particularly potassium) increase winterhardiness of alfalfa. Jung and Smith (33), in a greenhouse experiment with alfalfa using nutrient solutions, found that the percent survival and the amount of top growth increased when phosphorus was held at 80 pounds per acre and potassium was added up to 200 pounds per acre. Survival and top growth then decreased with increasing amounts of potassium. The maximum survival and yield came when potassium was held at 200 pounds per acre and 40 pounds of phosphorus per acre was applied. When phosphorus was increased to 80 pounds per acre, survival and yield decreased. The percent survival was constant when the ratio of potassium:phosphorus was 5:2.

Kuska (39) found that applications of a 4:1 ratio of phosphorus and potassium, with normal nitrogen (normal is not defined), markedly increased the winterhardiness and the total sugar content of winter wheat. These results are in opposition to most investigations which have the phosphorus:potassium ratio reversed. He also found that increased nitrogen decreased both sugars and winterhardiness, which agrees with the results of most investigators. In all cases, he had to apply phosphorus and potassium at greater rates than the nitrogen to get winterhardiness.

The influence of cool temperature (70-85° F) and warm temperature (85-100° F) interactions with nitrogen, phosphorus, potassium, calcium, magnesium and sulfur on Midland bermudagrass was checked by Lathapipat (41). A complete fertilizer treatment and a check treatment were also

included. The potassium, sulfur, calcium and magnesium treatments resulted in plant tissues being much higher in alcohol soluble sugars than the check, nitrogen and phosphorus treatments. The complete treatment was intermediate in alcohol soluble sugars. He also reported that each treatment was significant at the .05 level for cold temperatures.

Tifgreen bermudagrass (Cynodon dactylon X C. transvaalensis) was grown in nutrient solutions and then checked for coldhardiness by Reeves, McRee and Broadworth (77). They reported only a 1-2° C variation in coldhardiness, and concluded that within this range, the phosphorus:potassium ratio in the tissue influenced coldhardiness. A high phosphorus:potassium ratio increased winterkill. Tissue nitrogen had little effect except on the uptake of phosphorus and potassium. The greatest injury was found in high phosphorus treatments with no potassium. With a high phosphorus:potassium nutrient ratio there was considerable cold damage, while a high potassium:phosphorus ratio showed the greatest cold tolerance. Wolde-Tsadick (109) fertilized weeping lovegrass (Eragrostis curvula (Schrad.) Nees), switchgrass (Panicum virgatum L.), and big bluestem (Andropogon gerardi Vitman) and checked the effect on the total available carbohydrates (TAC). There was a significant increase in TAC in fertilized weeping lovegrass in September. However, by January there was no difference in coldhardiness in plants grown in the fertilized and unfertilized plots. There was no difference in the TAC of switchgrass and big bluestem as a result of fertilization.

Gilbert and Davis (21) found that nitrogen, phosphorus and potassium all played a role in the development of coldhardiness of Tifgreen and Tifdwarf (Cynodon spp.) bermudagrasses. A ratio of 4:1:4 nitrogen

phosphorus:potassium was the best. The nitrogen only treatment was the least hardy. When nitrogen levels were increased at a given level of phosphorus and potassium, winterhardiness increased.

Timothy was grown on phosphorus or potassium deficient soil with various rates of nitrogen, phosphorus and potassium by Suzuki (103). Water soluble carbohydrates were extracted from the plants. The water extract was then chromatographed through separation columns. He found that water soluble carbohydrates extracted from potassium deficient plants, unlike those deficient in nitrogen and phosphorus, showed an effluent pattern with an extremely low peak of long-chain fructosan and a relatively high peak of sugars. This indicated that potassium played an important role in the accumulation of long-chain fructosan. This study, when combined with those of Sakai (83) and Olien (73, 74), provides an explanation of the way potassium functions in coldhardiness.

It is apparent that nitrogen alone generally decreases winterhardiness, and that treatments with high levels of phosphorus in relation to nitrogen or potassium also result in lower coldhardiness. It is just as apparent that high levels of potassium, with or without the other major nutrients results in increased coldhardiness. It appears that a balanced fertilizer treatment with phosphorus being lower than nitrogen or potassium, and the potassium being applied late in the summer will increase coldhardiness.

From the studies reported here, it appears that coldhardiness and freezing injury are the result of complex interactions between chemical, physical and environmental factors.

CHAPTER III

MATERIALS AND METHODS

Field Experiments

An investigation to determine the effects of soil fertility on coldhardiness of common bermudagrass rhizomes was initiated in the spring of 1970 at the Agronomy Research Station near Perkins, Oklahoma. The experiment was conducted on an old stand of common bermudagrass. The experimental design was a randomized complete block with three replications. The soil type is a Eufaula loamy fine sand. The 27 fertility treatments as shown in Table I were applied on June 10, 1970.

Rhizomes were dug monthly from each plot from October, 1970, through April, 1971. The rhizomes were placed in a simple press (Figure 1) and a few drops of juice were extracted. The juice was caught in a Number 2010 Atago Hand Sugar Refractometer.

In the spring of 1971, the experiment was abandoned at Perkins, and a new one initiated on a long established stand of common bermudagrass on the main Agronomy Research Station at Stillwater, Oklahoma. The plots were on a Norge loam soil in a randomized complete block design with three replications. The same fertilizer treatments as used at Perkins were applied on April 12, 1971. Approximately 40 lbs. of additional nitrogen was accidently applied to the entire area during the month of April.

TABLE I

AMOUNTS OF ELEMENTAL N-P-K APPLIED IN KILOGRAMS PER HECTARE (POUNDS PER ACRE)

Kg/ha	(Lb/acre)	Kg/ha	(Lb/acre)	Kg/ha	(Lb/acre)
56-0-0	(50-0-0)	112-0-0	(100-0-0)	168-0-0	(150-0-0)
56-0-45	(50-0-40)	112-0-45	(100-0-40)	168-0-45	(150-0-40)
56-0-90	(50-0-80)	112-0-90	(100-0-80)	168-0-90	(150-0-80)
56-45-0	(50-40-0)	112-45-0	(100-40-0)	168-45-0	(150-40-0)
56-90-0	(50-80-0)	112-90-0	(100-80-0)	168-90-0	(150-80-0)
56-45-45	(50-40-40)	112-45-45	(100-40-40)	168-45-45	(150-40-40)
56-90-90	(50-80-80)	112-90-90	(100-80-80)	168-90-90	(150-80-80)
56-45-90	(50-40-80)	112-45-90	(100-40-80)	168-45-90	(150-40-80)
56-90-45	(50-80-40)	112-90-45	(100-80-40)	168-90-45	(150-80-40)

Figure 1. Photograph of Atago Hand Refractometer, Weksler Soil Thermometer, and Press Used to Extract Juice From Bermudagrass Rhizomes

Rhizomes were dug and the sugar percentages were determined from the Stillwater experiment during July, August, and September. In both of the tests, the sugar determinations were made on the first of the month. A Weksler Soil Thermometer was used to determine the soil temperature so adjustments could be made to the refractometer readings.

Killing Temperature Determinations

According to Levitt (46), several conditions must be met before a relatively constant frost killing temperature can be determined for any plant variety in a specific physiological state. These conditions are: (1) the plants must actually be frozen, not merely undercooled (air

movement will induce freezing); (2) freezing must be at a standard rate, (e.g. 2° C per hour); (3) a single freeze must be used for a standard length of time; (4) thawing must be at a standard rate; (5) conditions after thawing must be standardized (e.g. returning plants to hardening chamber (5° C) for 24 hours.

A Sherer-Gillette model 4608 M chest type growth chamber was used to freeze the rhizomes. A section of rhizome containing three nodes, with buds present, was termed a sprig. Nine sprigs from each replication of each fertility treatment were placed in a 25 x 150 mm test tube which was then sealed with a rubber stopper. Due to the possibility of uneven temperatures within the large freezer (over 50 cu. ft.), the test tubes were immersed in a mixture of ethanol and water (8:2 v/v) which had been brought to a predetermined temperature. When this mixture was frozen, it formed a thick slush which Dexter (15) had shown to be very good in preventing rapid temperature changes.

The mixture was held in an ordinary two gallon galvanized bucket. The test tubes were held in a one inch thick circle of styrofoam that had been cut to fit the mouth of the bucket. Holes were made to hold the tubes by simply pushing the tube through the styrofoam. The level of the ethanol mixture was adjusted to be above the level of the sprigs in the test tubes but not to touch the styrofoam. The mixture in the bucket started to freeze around the outside first. To prevent the ice from forming an insulating barrier, the tubes were momentarily lifted from the mixture and the ice crystals scraped from the sides of the bucket and the mixture thoroughly stirred at 30 minute intervals.

The sprigs were frozen for periods of 4, 8, 12, 16, 20, 24, and 48 hours, three, four, five and seven days at -2.2, -3.3, -4.4, -6.7, -7.8,

-8.9, and -10° C. Prior to freezing, the sprigs were hardened at 1.7° C for a minimum of seven days. Previous work has shown this temperature to be adequate to produce hardening (3, 5, 33).

After a killing temperature had been determined by using unfertilized sprigs, the technique was checked by making a test on 13 of the fertility treatments to determine if this temperature produced differences in the percent kill.

Three sets of seven hardened sprigs from each fertility treatment were placed in the test tubes as described earlier and placed in the ethanol-water mixture which had been previously cooled to 1.1° C. The temperature was lowered 2.2° C per hour until -8.9° C had been reached (the predetermined killing temperature). This temperature was maintained for two hours and then the entire ethanol-water mixture and tubes of sprigs were moved to a 1.7° C freezer and allowed to thaw. Sprigs harvested in each of three months, March, August, and September, were frozen.

Sprouting Investigations

The thawed sprigs were sprouted in moist vermiculite in a semi-pervious cellulose fiber container. The vermiculite was saturated and excess water was poured off. The sprigs were individually dipped into a fungicide solution /(trichloromethyl) thio/-4-cyclohexene-1, 2-dicarboximide (Captan) to discourage mold growth and then placed vertically in the vermiculite, with the bud tips pointing upward. No attempt was made to insure that equal amounts of various sprigs were either exposed or covered. However, a portion of each sprig was left exposed. Each container contained sprigs from four fertility treatments

(see Figure 2).

Figure 2. Photograph of Sprouting Sprigs in Cellulose Containers

The containers were placed in a Stults germinator held at a constant 20° C. The germinator had flourescent lights on three sides which were on eight hours and off 16 hours each day. Since previous work had shown that 20 days were required for complete sprouting, the sprouting tests were halted after 21 days and readings on number of leaves, number of roots, and length of roots were made. The locations of the leaves and roots, by node, were also recorded. The sprigs were inspected daily during the three weeks and the vermiculite was moistened as required. Occasionally mold became a problem, and the containers

were moistened with a Captan solution, instead of water, to control the mold.

Greenhouse Experiment

On October 15, 1971, circular plugs 8.9 cm in diameter and 15.2 cm deep were cut from an old stand of common bermudagrass. Since the plugs were taken from an overgrazed pasture, there was little leaf growth present. After washing away as much of the soil as possible, the plant material was then planted in sterile sand in 15.2 cm diameter clay pots.

The pots were placed in the greenhouse in a randomized complete block design having four treatments with 14 replications. The pots were watered with distilled water as often as required. At the end of four weeks, the plants had all made good top growth but were showing definite deficiency symptoms.

At this time, a new regime was initiated. A modified Hoagland's solution was used to apply four fertility treatments. Stock solutions were prepared by dissolving 236.16 grams of $Ca(NO_3)_2 \cdot 4\ H_2O$ and diluting to one liter for nitrogen, 25.21 grams of $Ca(H_2PO_4)_2 \cdot H_2O$ dissolved and diluted to one liter for phosphorus, 74.55 grams of KCl for Potassium, 101.5 grams of $MgSO_4$ for magnesium, 5.0 grams of iron sequestrene per liter for iron, and one ml per liter of a trace element mixture. This mixture was made by adding 2.86 grams of H_3BO_3, 1.81 grams of $MnCl_2 \cdot 4\ H_2O$, 0.22 grams of $ZnSO_4 \cdot 7\ H_2O$, 0.08 grams of $CuSO_4 \cdot 5\ H_2O$, and 0.02 grams of $H_2MoO_4 \cdot H_2O$ and diluting to one liter. The stock solutions were modified so that the ratios of elemental nitrogen, phosphorus and potassium were 4:1:6, 4:1:3, 1:2:1, and 4:0:0. In addition, magnesium, iron, and a complete mixture of trace elements were

added to each of these four treatments.

Fifty milliliters of the fertilizer solutions were applied to the respective pots once each week for 16 weeks. Prior to this application, the pots were leached with 500 ml of distilled water to prevent a salt accumulation. During the week, the plants were watered as needed with distilled water.

The plants made rapid growth the first two weeks after the addition of the nutrient solutions. A prolonged period of cloudy weather during the third and fourth weeks markedly reduced growth. Consequently, continuous flourescent lighting was placed over the pots for the rest of the growth period. During the ninth through the twelfth week, the leaf growth was clipped to a five centimeter height anytime it exceeded 10 cm to encourage stolon formation. During the last four weeks of the experiment, the plants were not clipped.

At this point, the plants were moved to a hardening chamber which was maintained at 1.1-1.7° C. The chamber was set to provide eight hours of light and 16 hours of darkness. The light was provided by a combination of incandescent and flourescent bulbs which produced approximately 3000 foot candles of illumination 61 cm from the bulbs. The plants were hardened under these conditions for four weeks. They were watered as required with distilled water.

At the end of the hardening period, a thermometer was inserted into one pot in each replication until it was in contact with the rhizomes. The thermometers were placed in such a fashion that they formed a flattened W, and in this position they gave a fairly representative sample of the temperatures in the growth chamber.

After the thermometers were placed, the plants were frozen in the

same manner as described earlier, with one exception. After the plants had been frozen at -8.9° C for two hours, the temperature was raised at the same rate it was lowered until 1.7° C was reached. The temperature was recorded each hour for each of the thermometers in the pots. An average of the 14 readings was used as the chamber temperature at any given hour. The plants were allowed to remain at 1.7° C for three days and were then returned to the greenhouse where the leaf height was clipped to a five centimeter height.

The plants were left in the greenhouse for five weeks and then evaluated as to the amount of freezing injury by measuring the amount of regrowth after clipping. During this period the plants were watered as needed with distilled water.

Laboratory Investigations

Electrolysis Study

The purpose of this study was to determine if electrolysis readings could be used rather than sprouting counts to measure freeze damage in bermudagrass rhizomes. Electrolysis has been used for some years to determine cell damage in plants, or to detect differences in coldhardiness (15, 17, 93). A modified system of Dexter's (15) was used.

At the time sprigs were dug for the sugar readings, a quantity was also dug for the sprouting and electrolysis tests. These sprigs were placed in (Glad) plastic sandwich bags. The bags were then placed in a cooler with the temperature maintained at 1.7° C until the sprigs were used.

The roots were trimmed from the sprigs and the soil and covering

membraneous non-green leaves were removed from the rhizomes. Various methods of washing, brushing and rubbing were tried in cleaning the sprigs. However, any method that got the sprigs clean enough for the electrolysis test also removed most of the buds. Consequently, the sprigs were cleaned by rubbing gently with the fingers, and peeling the scale-like leaves away. Since any appreciable amount of soil present on the rhizomes resulted in an inaccurate reading, great care was taken to insure that the rhizomes were clean.

About 30 minutes were required to clean approximately eight grams of sprigs and place them in a test tube for freezing. At the same time, sprigs were selected for the sprouting counts. Half of the remaining sprigs were placed in a test tube to be frozen along with the eight gram sample. The other half was placed in a test tube and stored at 1,7° C until after the freeze test. These sprigs were used as a pre-freeze sprouting check.

Occasionally after cleaning the eight grams for the electrolysis test, there would not be enough sprigs left to provide 14 sprigs for the sprouting counts. Several treatments throughout the test had only five sprigs each in the sprouting tests. Due to the lapse caused by the time required to clean the sprigs, it was impossible to get more material from the field without introducing an additional source of error. The data for these missing sprigs were reconstructed for statistical purposes by using Snedecor and Cochran's (98) missing plot formulas.

As each replication of the 27 fertility treatments was cleaned, it was frozen and thawed as explained previously. Following the freezing, the sprigs were placed in the germinator and the electrolysis test was started in the laboratory.

The sprigs from each plot were weighed and five milliters of deionized distilled water per gram of sprigs was added to the test tubes. The tubes were then placed in a refrigerator set at 0° C and allowed to stand overnight. Previous work by Dexter (15) showed that after 10 hours little, if any, exosmosis occurred. This allowed the electrolytes from the freeze damaged cells to escape into the water. After soaking overnight, the solution was removed and a current passed through it using a Yellow Springs Instrument Co. Model 31 Conductivity Bridge. The greater the cell damage, the more electrolytes escaped into the water and the greater the current which passed through the solution. Following the electrolysis reading, the solution was returned to the sprigs and they were placed in a boiling water bath for 10 minutes to kill all of the cells. After boiling, the tubes were again placed in the refrigerator at 0° C and allowed to stand overnight. The solution was again poured from the tubes and another electrolysis reading was made. The difference between the two readings was used as a measure of the amount of freezing injury. The greater the difference, the less damage. Electrolysis readings were made for samples dug during March and August.

Sugar Determinations

Since the amount of rhizomes needed to make sugar determinations with the refractometer was too great to be used with the greenhouse material, a different method of determining sugar content had to be developed.

The anthrone technique as modified by Roe (79, 80) provided a quicker method of determination than had been previously used and also provided a reagent which could be stored for as long as two weeks.

Hudson (unpub. data, Hudson, Billy G., Dept. of Biochemistry, Oklahoma State University, 1972) further modified Roe's technique by eliminating the need for precipitating the proteins and reducing the amount of materials required by half. Hudson's technique was further modified and used in this investigation.

To prepare 500 ml of anthrone reagent, 360 ml of concentrated H_2SO_4 was carefully added to 140 ml of distilled water in a one liter Erlenmeyer flask. After the boiling had subsided, 0.25 gm of anthrone and 5.0 gm of thiourea were added, with swirling. The reagent could be stored in the refrigerator for about three weeks.

A small section of rhizome was removed from each pot just prior to freezing. The section was placed in a stoppered test tube and stored at 0° C. An attempt was made to obtain a section from the same node of a newly formed rhizome, although this was not always possible. The sand in the pots was disturbed as little as possible. Rhizomes were usually obtained by scraping aside 5-7 cm of sand to a depth of 2-4 cm.

A two milligram section from each of these rhizomes was weighed on a Mettler Analytical Balance. Extreme care was taken to insure that exactly two milligrams was obtained. This was done by slicing off very small sections with a razor blade if the sample was too heavy, or by adding small pieces, if it was too light. By exchanging, adding or subtracting these smaller pieces, the exact amount could be obtained. The test tubes containing the rhizomes were kept in an ice bath except when sections were actually being cut and weighed.

Each of the weighed samples of rhizomes was placed in an 18 x 150 mm test tube that contained 10 ml of 80% ethanol and a boiling stone. The test tubes were then placed in a water bath heated to 32.2° C until

the alcohol had evaporated.

One milliliter of this alcohol soluble sugar solution from each sample was added to another 18 x 150 mm test tube. Five milliliters of the anthrone reagent was added to each tube. Each tube was capped with a glass marble and placed in a boiling water bath for 15 minutes. When removed from the water bath, the tubes were immersed in a cold water bath for about 20 minutes. A series of standard sugar solutions was processed at the same time. After cooling, the solutions were transferred to a crystal tube and the absorbance read at 630 mμ in a Beckman Model DB spectrophotometer.

Two individual anthrone tests were run on different days using different sections of the original rhizome along with a group of standard sugar solutions. The standard curve was computed by averaging the readings obtained from these two tests of the standard sugar solutions.

Sugar Identification

After the second anthrone test had been completed, a section of rhizome from 2.5-7.5 cm long remained from each pot. All of these sections were bulked and dropped into a beaker containing 200 ml of 80% ethanol which was immersed in a boiling water bath. After about half of the ethanol had boiled away, the liquid was poured off of the rhizomes, boiling stones were added to the extract, and it was returned to the waterbath. The remainder of the ethanol was evaporated and the remaining water fraction containing the alcohol soluble sugars was further evaporated until approximately two milliliters of the sugar solution remained. This sample was placed in a small vial and stored at 0° C.

Standard commercial (Kieselguhr) Silica Gel G, 20 x 20 cm thin layer plates were used in these chromatographic investigations. Plates are referred to as being "Active" or "Inactive." The active plates were heated for three hours at 105-110° C in an oven prior to spotting. The inactive plates were simply used as they came from the dessicator.

The standard sugar solutions were made by dissolving one mg of sugar in 9.9 ml of distilled water. The pentoses were applied at 30 μg per spot, and the other sugars and the unknown were applied at 40 μg per spot with a micro-syringe, unless otherwise stated. The origin was 1.5-2.0 cm above the bottom edge of the plate. The solvent was placed in the tank at least 30 minutes before the plates were added to allow the air in the tank to become saturated.

After the plates were developed and air dried, they were sprayed with the detecting reagent and heated in an oven at a temperature and a period of time recommended for the reagent.

The following solvent systems were used to successfully separate the sugars:

1. one normal butanol-glacial acetic acid-ethyl ether-water (9:6:3:1 v/v)
2. pyridine-ethyl acetate-glacial acetic acid-water (5:5:3:1 v/v) (19)
3. methyl ethyl ketone-glacial acetic acid-methanol (60:20:20 v/v)
4. benzene-glacial acetic acid-methanol (20:20:60 v/v)
5. butanol-acetone-water (40:50:10 v/v)

The reagents used in successful identifications were:

1. ethanol-sulfuric acid-anisaldehyde (18:1:1 v/v)

2. resorcinol-trichloroacetic acid (one part by volume of an alcoholic-resorcinol solution (0.2 gm resorcinol dissolved in 100 ml ethanol) is mixed with one part by volume of an aqueous 20% solution of trichloroacetic acid) (107).

Solvent system number two was used with Whatman No. 1 Paper in an ascending tank in order to separate some sugars which had been difficult to separate on the thin layer plates.

Some plates were also saturated with a 0.1 N boric acid solution prior to use. According to Stahl and Kaltenbach (99), manually prepared plates using this boric acid solution instead of water were particularly useful for separation of mixtures of sugars.

The commercially prepared plates were sprayed in a hood, as if the boric acid was a reagent, until the silica gel layer was thoroughly saturated. Care had to be taken not to overspray, as a continued "soaking" resulted in the binder separating from the glass plate rendering the plate useless. If the plate was sprayed thoroughly and quickly, the draft from the hood dried the plate rapidly enough to prevent this separation from taking place.

Solvent system number one was used to develop Plate 1, an inactive plate, for four hours and 30 minutes. The plate was sprayed with reagent number one and was heated for three minutes at 100° C to bring out the spots.

Another inactive plate, Plate 2, was developed in the same solvent for five hours and 30 minutes, developed with the same reagent, and heated for 3½ minutes at 100° C.

Plate 3, an active plate, was developed 2½ hours in solvent number three after being spotted with 30 μg of the unknown and several

standards. After developing, the plate was air dried, sprayed with reagent number one and heated at 100° C for five minutes. This test was repeated with Plate 4 except that 30 μg of the unknown and glucose was used, 20 μg of maltose and arabinose and 10 μg of sucrose. The plate was also heated after spraying for seven minutes rather than five minutes.

Sucrose and fructose were spotted at 20 μg on the lower right-hand corner and the upper left-hand corner of Plate 5, an active plate. Thirty μg of the unknown was spotted in the lower left-hand corner. The plate was developed first in solvent number four for 1½ hours, air dried, tipped 90° to the left, and developed in solvent number five for another 1½ hours. After drying, the plate was sprayed with reagent number one and developed at 105° C for three minutes.

Plate 6 was saturated with boric acid solution, then activated and spotted with 30 μg of the unknown in the lower left corner and 20 μg of sucrose, fructose and glucose in the lower right and upper left corners. It was then developed two hours and 20 minutes in solvent number five, air dried, tipped 90° to the left, and developed one hour and 25 minutes in solvent number three. After drying, it was sprayed with reagent number two and heated at 105-110° C for 12 minutes. The standards appeared very faintly and the unknown was not discernable. The plate was then sprayed with reagent number one and heated at 105° C for five minutes.

Thirty μg of the unknown along with glucose, galactose, and mannose were spotted on Whatman No. 1 paper and developed in solvent number two for 5½ hours. The paper was then air dried and sprayed with reagent number one and heated at 100° C for three minutes.

After as many sugars as possible had been determined with thin layer or paper chromatography, two samples, one hydrolyzed and one unhydrolyzed, were processed through a Technicon Auto-analyser using the system developed by Lee, McKelvy and Lang (42).

One hundred μg of the alcohol soluble sugar solution was hydrolyzed at 100° C with 0.8 ml of two N sulfuric acid for four hours in a sealed test tube. The neutral sugars and amino sugars were separated on Dowex 1 and Dowex 50 resin columns. After the separation, 10 ml of water was added to the sample and then a two ml fraction of this sample was processed through the resin columns, and the auto-analyzer without being hydrolyzed.

CHAPTER IV

RESULTS AND DISCUSSION

Field Experiments

The fertility treatments used in these field experiments had no effect on the sugar content of the bermuda rhizomes. Two explanations for these results can be suggested at this time. The area selected for the experiment seemed to be uniform based upon the appearance of the bermudagrass and the slope. By checking with Experiment Station personnel and faculty members, it was determined that the area had received no fertility treatments for the past 15 years. However, after the experiment was concluded, it was found that this area had been the site of a fertility test prior to that time. Past fertility treatments could thus have obscured the effects of the recently applied fertilizer on the sugar content of the rhizomes.

The second possible explanation is that the fertility treatments used were below the threshold of response for influencing sugar content of the rhizomes. This possibility is suggested by the work of Gilbert and Davis (21) which was discussed earlier (page 19).

The major factor influencing the sugar levels was the month in which the readings were made. The sugar percentages for the seven months in which readings were taken are shown in Figure 3. Rain during the first week of May and June prevented the collection of data for

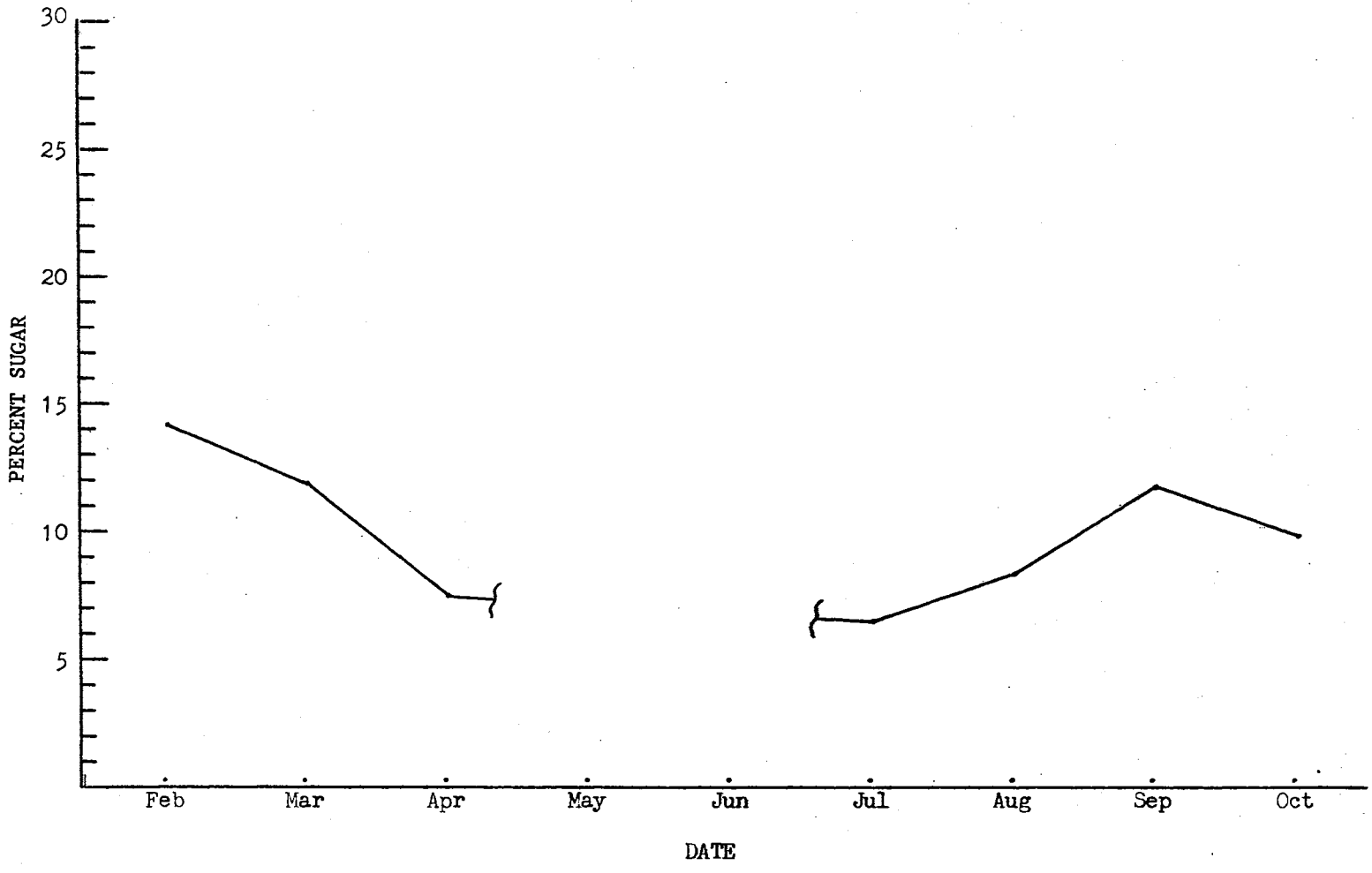

Figure 3. Percent Sugar Found in Bermudagrass Rhizomes for Seven Months of a Nine-Month Period

those months.

With the exception of the October reading, the data agree with many other researchers who have worked in this field (7, 34, 37, 50, 56, 75, 92, 108). The sugar content is highest during the winter months and lowest during the summer. The low October reading could possibly be accounted for by a period of unusually warm weather during the last week in September. The temperature for the last five days of September averaged 2.8° C higher than the first five days of October. The minimum temperatures of this period in September were also an average of over 6.1° C warmer than the average minimums for the October period. This period of high temperatures could have resulted in a conversion of sugars to starch just prior to the time the October sugar readings were taken.

The differences in sugar readings between replications were also significant at the 1% level as was the month x replication interaction. The analysis of variance for the field sugar readings is given in the Appendix, Table VII, page 99.

Killing Temperature Determinations

In the initial attempts to lower the temperature of the ethanol-water mixture to form a protective slush, it was found that it was sometimes an hour, or more, before the temperature started to fall in this mixture. However, once the temperature started down, it fell steadily at the rate of 1.1-2.2° C per hour.

It was important that a fresh mixture of ethanol-water be made prior to each freezing test, and that distilled water be used. It was found that the temperature in the mixture lowered at a steady rate and

then stopped at a level above the temperature recorded in the chamber. The mixture remained at this "plateau" for several hours before starting to fall again. Further investigation revealed that the location of the "plateau" temperature, and the length of time the mixture stayed at this level, varied with the ratio of ethanol and water, and with the purity of the water.

The tests started at a temperature of 4.4° C and went to a low of -10° C. The idea was to locate a time and temperature which would kill 50% of the sprigs from the unfertilized plots in the field. Testing times were initially 16, 20, 24, 36, 48, and 72 hours, and four, five, six, and seven days. The percent kill varied from 45% at 4.4° C for seven days, to 96% at -10° C for 16 hours. A temperature of -8.9° C for a two-hour period was selected since it caused approximately a 50% kill of the unfertilized sprigs.

Two replications of 13 representative fertility treatments were frozen at -8.9° C for a two-hour period. The results varied from a complete kill of sprigs in two treatments in each replication, to no damage to sprigs from one treatment in one replication and from two treatments in the other. The treatments used and percent kill found during this preliminary investigation are shown in Table II.

The variations within this preliminary test indicated the freezing temperatures and technique were satisfactory and the testing of the entire experiment was started.

Sprouting Investigations

During the period of time the killing temperature was being determined, a preliminary investigation was also being conducted to

TABLE II

EFFECT OF FERTILITY TREATMENTS IN THE FIELD ON THE SURVIVAL OF BERMUDAGRASS RHIZOMES SUBJECTED TO A FREEZING TEMPERATURE OF -8.9° C FOR A TWO-HOUR PERIOD

Fertility Treatment Elemental N-P-K		Rhizomes Percent Kill		
Kg/ha	(Lb/acre)	Rep 1	Rep 2	Avg.
56-0-0	(50-0-0)	66	22	44
56-45-0	(50-40-0)	12	0	6
56-45-45	(50-40-40)	100	100	100
56-90-90	(50-80-80)	88	66	77
56-90-45	(50-80-40)	66	66	66
112-0-90	(100-0-80)	0	0	0
112-90-90	(100-80-80)	88	66	77
112-90-45	(100-80-40)	66	89	77
168-0-45	(150-0-40)	22	22	22
168-45-0	(150-40-0)	100	89	95
168-45-45	(150-40-40)	22	88	55
168-45-90	(150-40-80)	22	66	44
168-90-45	(150-80-40)	66	100	83

determine the length of time the sprigs had to remain in the germinator to insure maximum sprouting. It was found that after 21 days all sprouting was complete. A sprig was considered live when it had at least one leaf and one root present at any of the three nodes. The leaf and root did not have to be at the same node.

A statistical analysis of the number of live sprigs showed no significant differences among fertility treatments in the main experiment. There was, however, a difference among effects due to months, freezing, and the interaction between months and freezing, all of which were significant at the 1% level.

The pre-freeze sprigs were those which had been harvested from a plot and sprouted without being frozen. The post-freeze sprigs were those harvested from the same plot, at the same time, as the pre-freeze sprigs and frozen at -8.9° C for two hours before being thawed and sprouted. The number of live sprigs from the pre- and post-freeze treatments, by months, is presented in Figure 4, while the analysis of variance for this portion of the experiment is presented in the Appendix, Table VIII, page 100.

The number of pre-freeze live sprigs from plots harvested in September numbered 16.6. This was a 298% increase over the low number of pre-freeze live sprigs, 5.6, which was found in the rhizomes harvested in March. The September count was also approximately 17% greater than the August count. There was very little difference in the post-freeze number of live sprigs.

The number of live sprigs for the pre-freeze treatment appeared to be increasing steadily as fall approached. This could be expected due to the probability that more nutrients were available as stored food

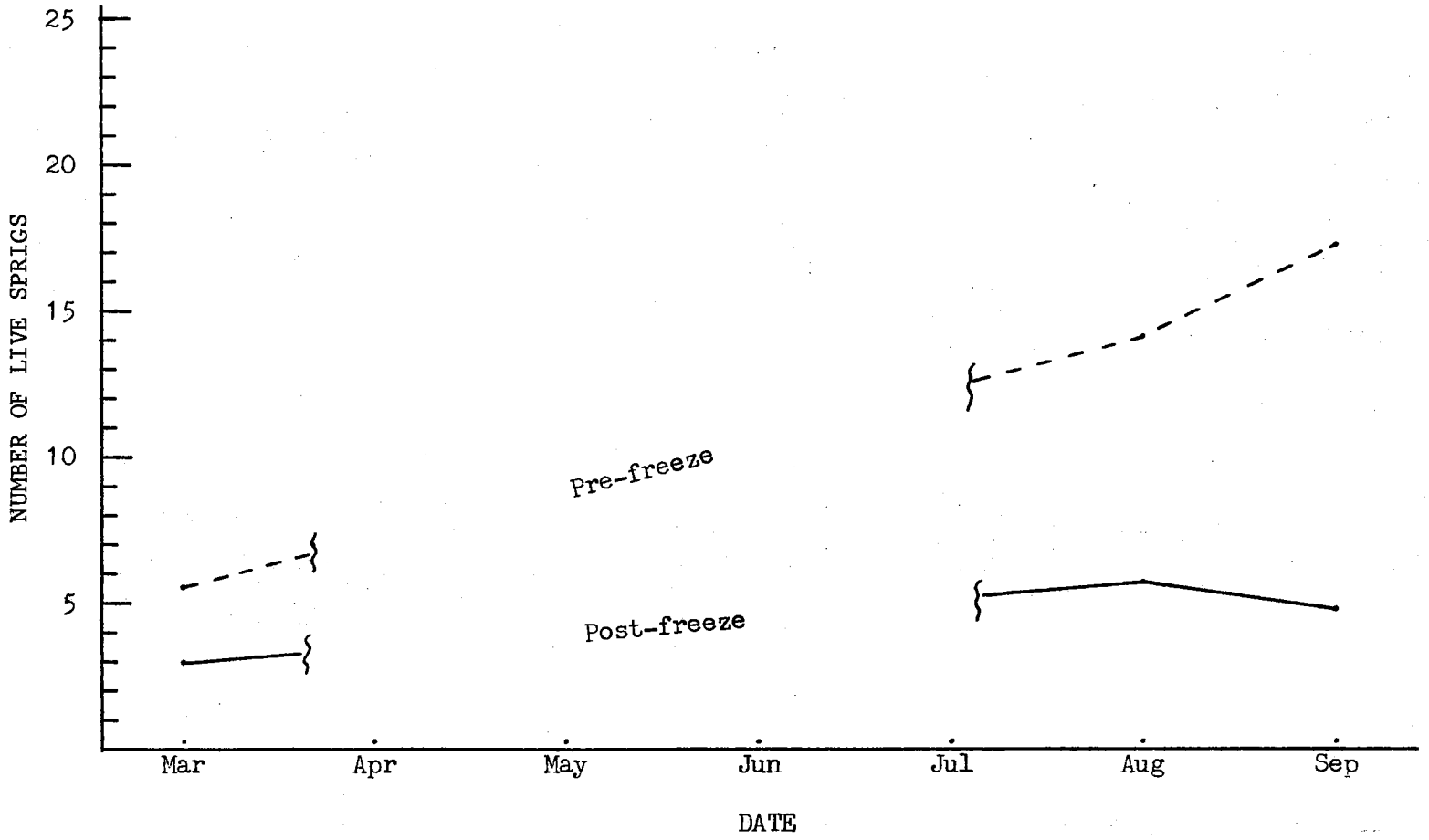

Figure 4. Number of Live Sprigs Harvested in Three Different Months, Pre- and Post-Freeze

reserves as the season progressed.

When the sprigs for March were dug, the killing temperature had not been determined. Consequently, these sprigs were stored from March until July at 1.7° C before they were tested. There was some hesitation about including these sprigs in the test. However, under close examination the sprigs appeared to be alive and uninjured. The plastic bags seemed to have prevented dehydration. When removed from the cooler, moisture would condense on the inside of the bags in a few seconds. The sprigs would "snap," or break, easily. While working with the Soil Conservation Service, the author found this snap test was considered a reliable test by farmers and sprigging contractors to check the viability of sprigs being used for field plantings. The dead sprigs would bend completely together without breaking. A microscopic examination might have shown that there were sufficient dead, or damaged, cells to have influenced the results obtained in this investigation. This possibility should be kept in mind when considering the following data.

From the pre-freeze data shown in Figure 4, it appears that the later in the season the sprigs are dug prior to freezing, the greater the number of surviving sprigs.

Basic botany and crop courses teach that a rhizome node is the location of the meristematic tissue which usually gives rise to both leaves and roots. As the investigation progressed, it became apparent that there were a great many sprigs that had either leaves or roots but not both.

The number of sprigs that had leaves without roots, and roots without leaves was analyzed. The analysis of variance for these factors is presented in the Appendix, Tables IX and X respectively, pages 101 and

102.

The interaction between freeze and rates of phosphorus was significant at the 5% level. These responses are presented in Figure 5. The best result, a leaf with every root, was found after freezing with no phosphorus.

After it was found that this area had been the location of an old fertility experiment, soil samples were taken from untreated areas adjacent to the test. An analysis of these samples showed phosphorus levels which varied from 179-246 kilograms of available phosphorus per hectare.

The worst post-freeze result, 0.30 of the roots without leaves, was obtained at the 112 kg per hectare phosphorus level, which was also about the same as the pre-freeze treatment with no phosphorus. Fifty-six kilograms per hectare of phosphorus significantly reduced the number of roots without leaves for the pre-freeze treatment, while the 112 kg rate once again resulted in an increase in roots without leaves (i.e. the 56 kg rate increased the number of completely sprouting sprigs, those with both a leaf and a root; while the 112 kg rate damaged the sprig in some manner so that the leaves failed to sprout).

The interaction between freeze and month was significant at the 1% level. March was the worst month having the greatest number of leaves without roots in both freezing treatments. The pre-freeze September sprigs were most viable and improved significantly over the August sprigs, (i.e. the September pre-freeze sprigs had the best sprouting, nearly every root having a leaf). On the other hand, the post-freeze sprigs were just the opposite having more roots without leaves in September than in August. These responses are presented in Figure 6.

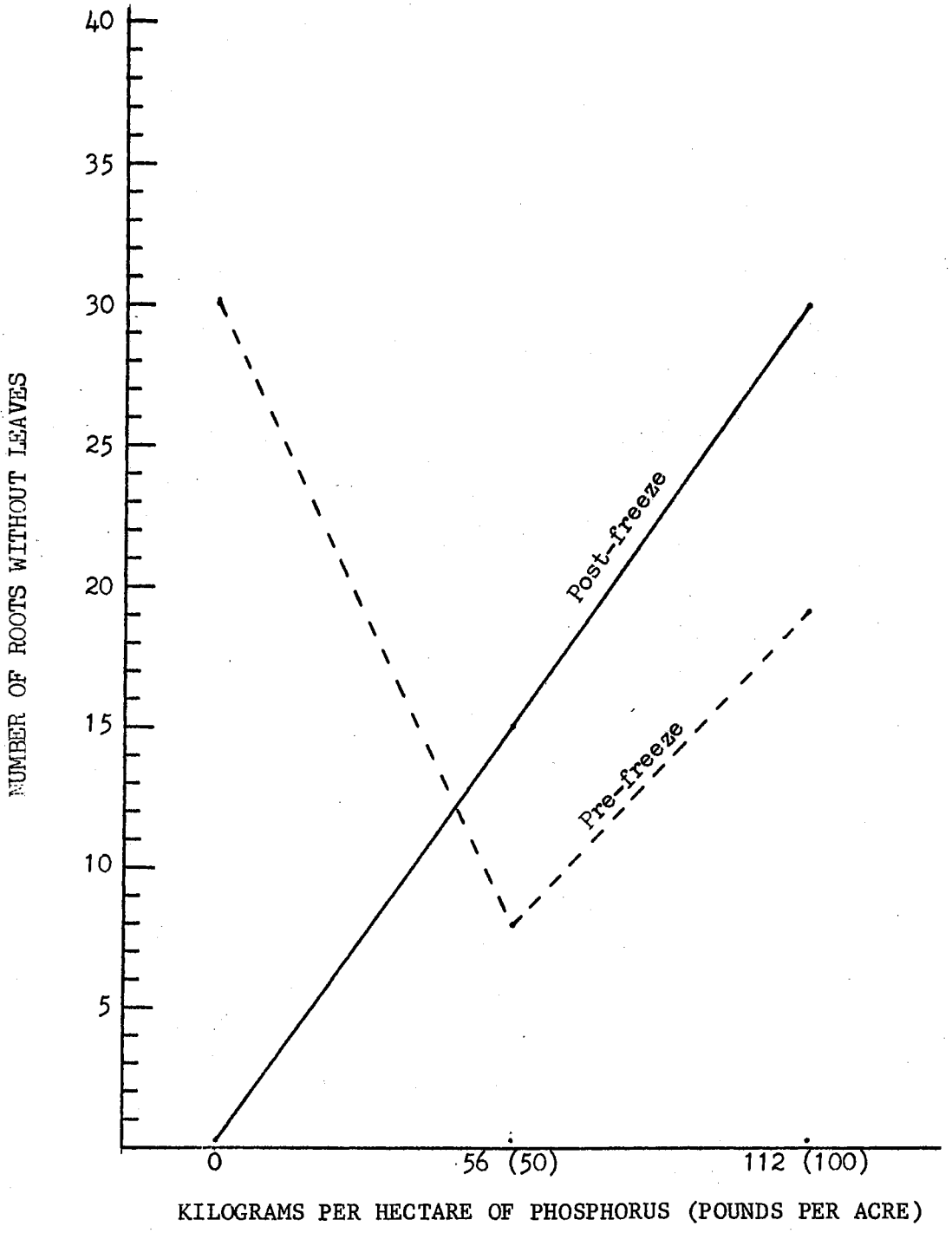

Figure 5. Number of Roots Without Leaves in Bermudagrass Sprigs Grown at Three Levels of Phosphorus, Pre- and Post-Freeze

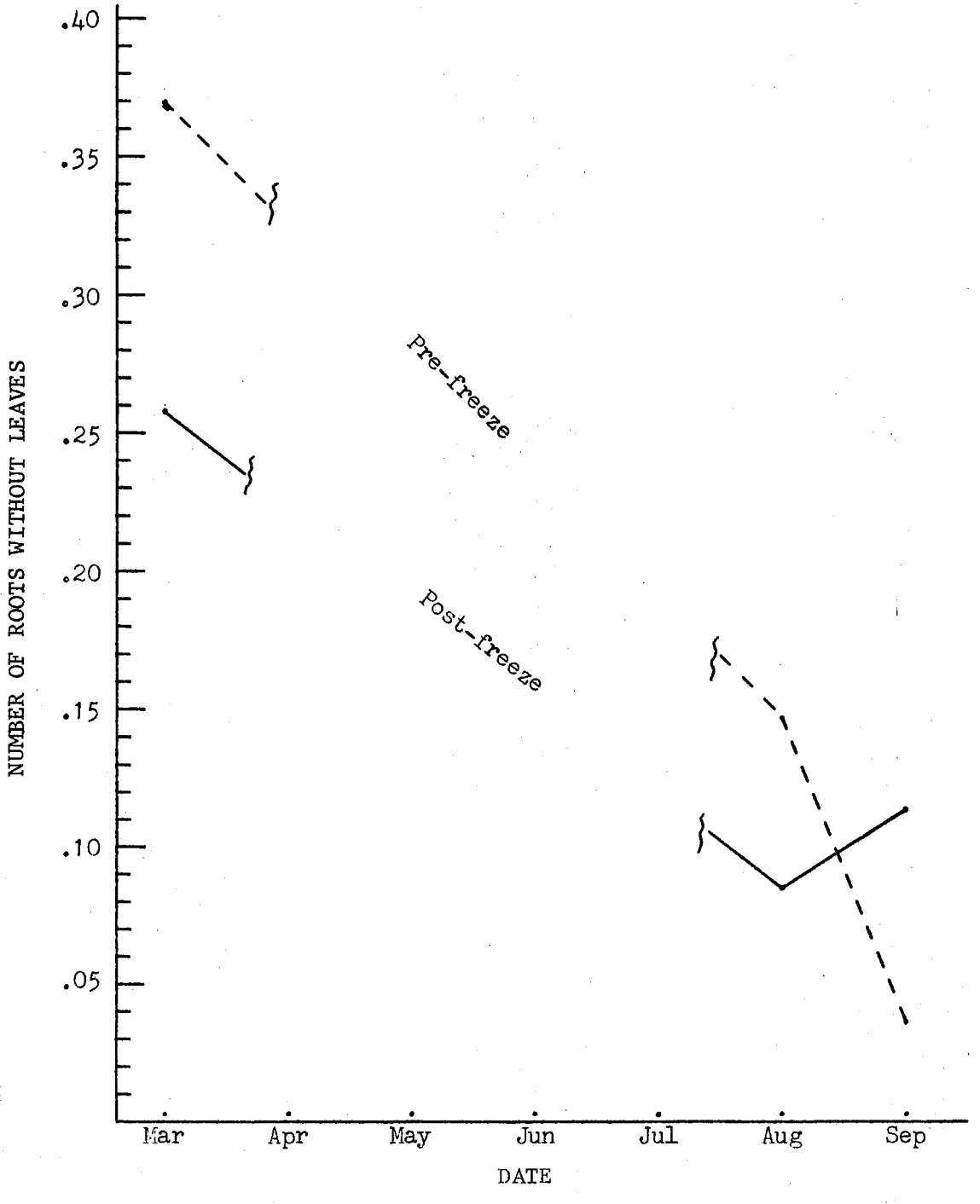

Figure 6. Number of Roots Without Leaves in Bermudagrass Sprigs Harvested in Three Different Months, Pre- and Post-Freeze

The number of leaves without roots showed much greater variation than did the roots without leaves. The number of leaves without roots was influenced by the date collected and the freezing treatments. There was also an interaction between the months and the freezing treatments. The variations between month, freezing treatments and the month by freeze interaction were all significant at the 1% level. This information is presented in Figure 7.

For the pre-freeze sprigs, the number of leaves without roots increased from March to August and then decreased sharply in September. This would indicate that prior to freezing some factor might have been present in the sprigs throughout most of the growing season which prevented root formation. At some time during August, this factor was greatly reduced so that most of the September sprigs produced both leaves and roots. This factor could have been influenced by temperature since the post-freeze sprigs improved (produced both leaves and roots) steadily from March to September. However, if such an inhibition occurred, it must have been influenced by more than temperature since the sprigs were all frozen at the same temperature and yet there was such a change from March to September. From August to September there was a rapid improvement (both roots and leaves formed) with 40% of the total decrease in leaves without roots occurring in this 30-day period. The leaves without roots also had a much wider variation between the pre-freeze and the post-freeze treatments than the variation between these treatments for the roots without leaves.

The pre-freeze sprigs collected in March had 9.5% more leaves without roots than the post-freeze sprigs. However, in August the pre-freeze sprigs had a much larger number of leaves without roots (40.2%),

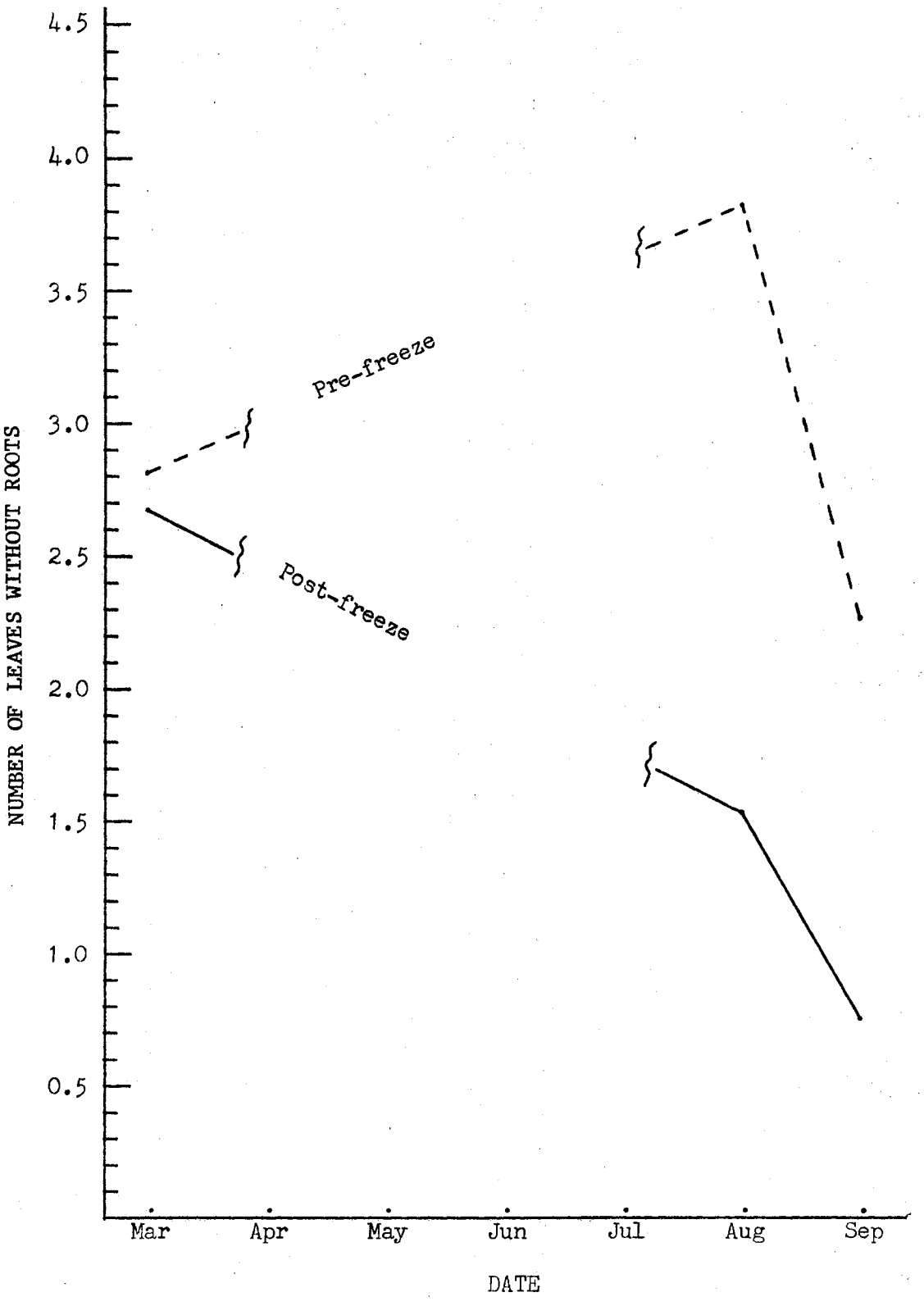

Figure 7. Number of Leaves Without Roots in Bermudagrass Sprigs Harvested in Three Different Months, Pre- and Post-Freeze

an increase over March of 30.7%. In September, the difference between the two treatments was 32.2%, an increase over March of 22.7%, but a reduction in the number of the leaves without roots over August of 7.6%.

The pre-freeze sprigs had over 31% more sprigs without leaves in August than they did in March, however, this number fell by 60.8% in September to a point 9.5% lower than March, i.e. the pre-freeze sprigs had a sharp decrease in complete sprouting (both a root and a leaf) from March to August. However, from August to September this decrease in complete sprouting was reversed and complete sprouting increased over August by nearly 61%, which was a 9.5% improvement over the March sprigs. The post-freeze sprigs had the same rapid drop from August to September. The post-freeze sprigs also improved consistently each month rather than having poorer complete sprouting as did the pre-freeze August sprigs.

When the actual number of leaves or roots in Figures 5, 6, and 7 are compared, it seems that they are insignificant. The numbers in these three figures are based on the total number of sprigs tested. However, if all of the dead sprigs are eliminated and only live sprigs which have a root present without a leaf, or a leaf present without a root are compared, the significance is immediately apparent as is shown in Table III. When nearly 93% of the live sprigs have leaves present without roots, it would seem that some factor is adversely influencing root formation.

A summarization of the numbers of leaves and roots present at each node for the entire study is presented in Table IV. There were 375 sprigs which were dead because roots did not form at any of the three nodes. In contrast, there were only 27 sprigs dead because of no

TABLE III

THE NUMBER OF LIVE BERMUDAGRASS SPRIGS (LS), ROOTS WITHOUT LEAVES (R w/o L), LEAVES WITHOUT ROOTS (L w/o R), AND THE PERCENT OF LIVE SPRIGS (% LS), PRE- AND POST-FREEZE FOR THREE DIFFERENT MONTHS

Month Harvested	PRE-FREEZE					POST-FREEZE				
	LS	R w/o L	% LS	L w/o R	% LS	LS	R w/o L	% LS	L w/o R	% LS
March	5.6	0.4	7.1	2.9	51.8	2.8	0.2	7.1	2.6	92.8
August	13.7	0.2	1.4	3.8	27.7	5.3	0.1	1.9	1.5	28.3
September	16.4	0.0	0.0	2.3	14.0	4.0	0.1	2.5	0.7	17.5

TABLE IV

NUMBER OF BERMUDAGRASS SPRIGS WITH LEAVES AND ROOTS PRESENT OR ABSENT AT EACH OF THE THREE NODES. R = ROOT, L = LEAF, 1 = ABSENT, 2 = PRESENT; TOP, MIDDLE AND BOTTOM NODES ARE DENOTED IN THIS ORDER FROM LEFT TO RIGHT*

	R111	R112	R121	R122	R211	R212	R221	R222	L Total	% of Total
L111	1553	0	23	1	1	1	1	0	1580	48.6
L112	3	7	3	0	17	1	6	1	37	1.1
L121	180	0	194	1	9	7	32	6	423	13.0
L122	16	2	58	16	34	4	49	13	179	5.5
L211	16	0	7	0	7	1	2	0	33	1.0
L212	44	3	11	0	13	92	4	9	167	5.2
L221	44	1	140	1	20	1	43	10	250	7.7
L222	72	11	175	17	89	119	96	75	580	17.9
R Total	1928	24	611	36	190	226	233	114	3249	
% of Total	59.3	0.7	18.8	1.1	5.8	6.9	7.2	3.5		

* e.g. In left hand column above, entry L221 means: Number of sprigs on which leaf on 1st and 2nd node is present and absent on the 3rd node. In the row across the top of the table, entry R112 means: Number of sprigs on which root on 1st and 2nd node is absent, but present on the 3rd node of the sprig. For this example one would find only one sprig that was coded L221, R112.

leaves. This is a total of 348 sprigs which would have been alive if a root had formed at any node, or 92.8% of the total of dead sprigs resulted from the absence of leaves or roots when one was present and the other absent.

A closer examination of this table shows some definite trends which are summarized in Table V.

TABLE V

PERCENT OF LEAVES AND ROOTS PRESENT OR ABSENT AT THE TOP, MIDDLE AND BOTTOM NODES FOR ALL BERMUDAGRASS SPRIGS, EXCLUSIVE OF THE SPRIGS WITH ALL ROOTS AND ALL LEAVES ABSENT

	LEAF		ROOT	
NODE	% Absent	% Present	% Absent	% Present
Top	39.3	60.7	61.7	38.3
Middle	15.6	84.4	48.0	52.0
Bottom	43.2	56.8	83.0	17.0

It appeared that the middle node of the bermudagrass sprigs arranged vertically in the germinator had an advantage over either the top or bottom nodes for both leaf and root production. It also seemed that the bottom node was the worst with the highest percent dead leaves and roots and also the smallest number of live leaves and roots. Particularly noticeable was the small number of live roots.

These data would suggest that the technique of standing the sprigs

on end in the germinating medium could have caused part of these differences. There is the possibility that the additional moisture in the vermiculite could have adversely influenced the bottom node, or that the vertical position could have caused the translocation of a plant hormone, or some other factor, in the rhizome which resulted in a lower viability of the bottom node. The only obvious advantage of the middle node was the fact that there was undisturbed tissue above and below, past another node. Both end nodes had undisturbed tissue only on one side. The area of damaged cells could possibly have influenced the meristematic tissue at the end nodes, although in what manner the author cannot explain.

The difference in the number of leaves and roots was also highly significant between months and freeze as shown in Figure 8. Once again the number of leaves and roots in August and September for the pre-freeze sprigs was much greater than the March sprigs. The number of roots and leaves on the pre-freeze sprigs also showed a sharp increase from August to September.

The differences due to the pre- and post-freeze treatments also increased considerably from March to September. The March post-freeze sprigs had 41% fewer leaves and roots than did the pre-freeze sprigs. This number had increased by 27% by August, and by September the difference was 36% greater than in March. The same relationship held true for the post-freeze sprigs, although the difference was not as great as in the pre-freeze group.

These data would indicate that rhizome viability increases as the season progresses. However, after a freeze, the number of leaves and roots formed on sprouting rhizomes is greatly reduced, particularly in

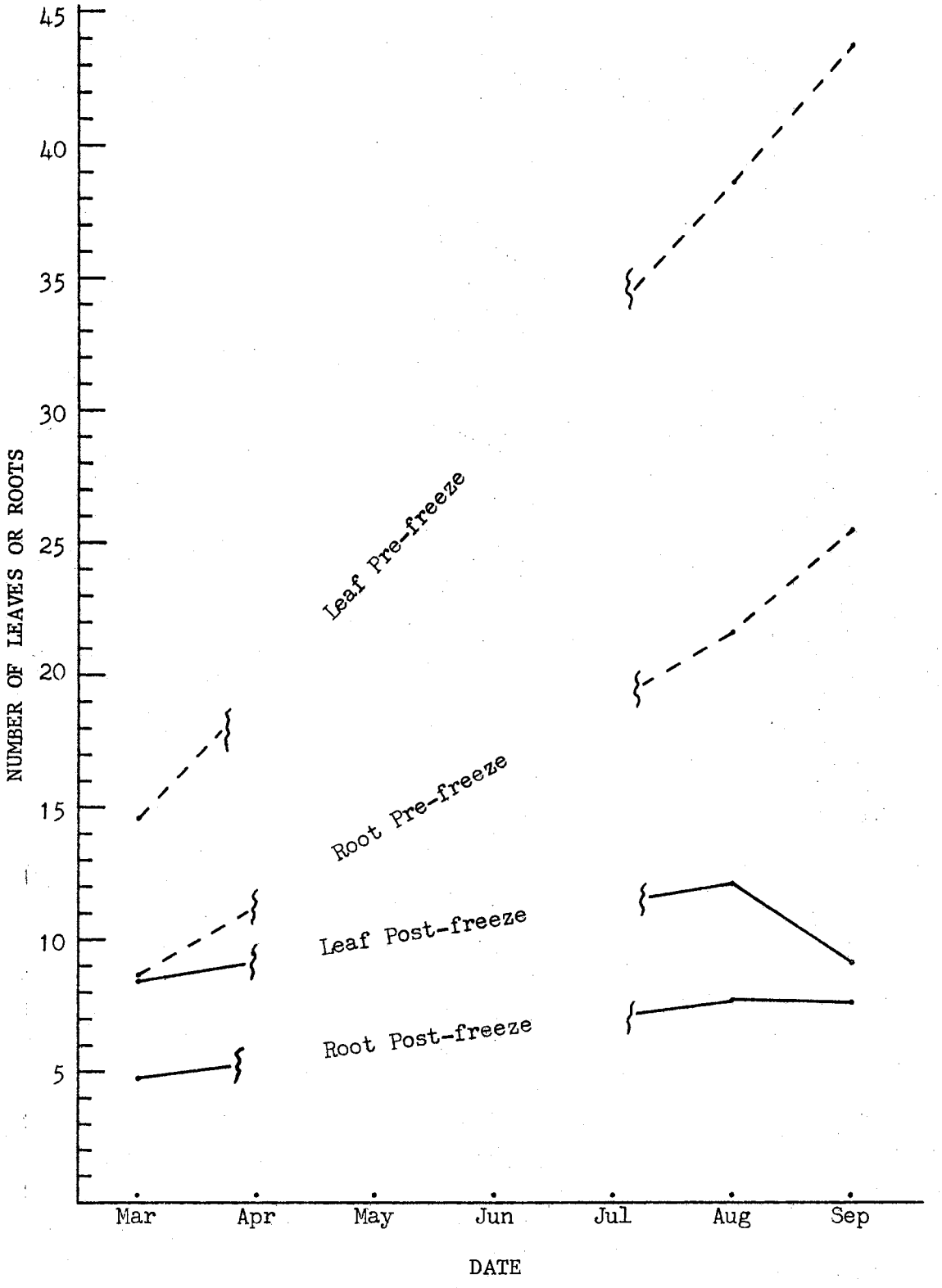

Figure 8. Number of Leaves and Roots in Bermudagrass Sprigs Harvested in Three Different Months, Pre- and Post-Freeze

the case of the leaves, which were nearly 20% fewer in number in September than in August. The post-freeze sprigs also showed a much smaller change from month to month than did the pre-freeze sprigs.

At the present time, no adequate explanation for the difference in the number of leaves and roots which occurred in this study can be given by the author, or by any of the plant physiologists with whom this subject was discussed.

Root Length

In addition to the above data, the length of the longest root at each node was measured to the closest centimeter. The pre-freeze sprigs had longer roots in August and September than they had in March, as shown in Figure 9. The lengths in September jumped sharply above the August lengths increasing by nearly 39% during this period. The lengths increased from March to September by over 289%.

The lengths of the post-freeze roots did not show such a rapid increase, differing only slightly from March to September. The spread between the pre- and post-freeze sprigs also widened as fall approached, increasing by 234% in August and another 104% in September. These data indicate that root growth is severely restricted following a freeze. It also indicates that sprigs dug in the late fall prior to a freeze, and planted, would produce the longest roots. This possibility could be explained if late in the fall sprigs contained more stored nutrients than those harvested in March.

The location of the roots also had a marked effect on their length as shown in Figure 10. The top and bottom nodes from the March and August sprigs had much shorter roots than did the middle nodes for both

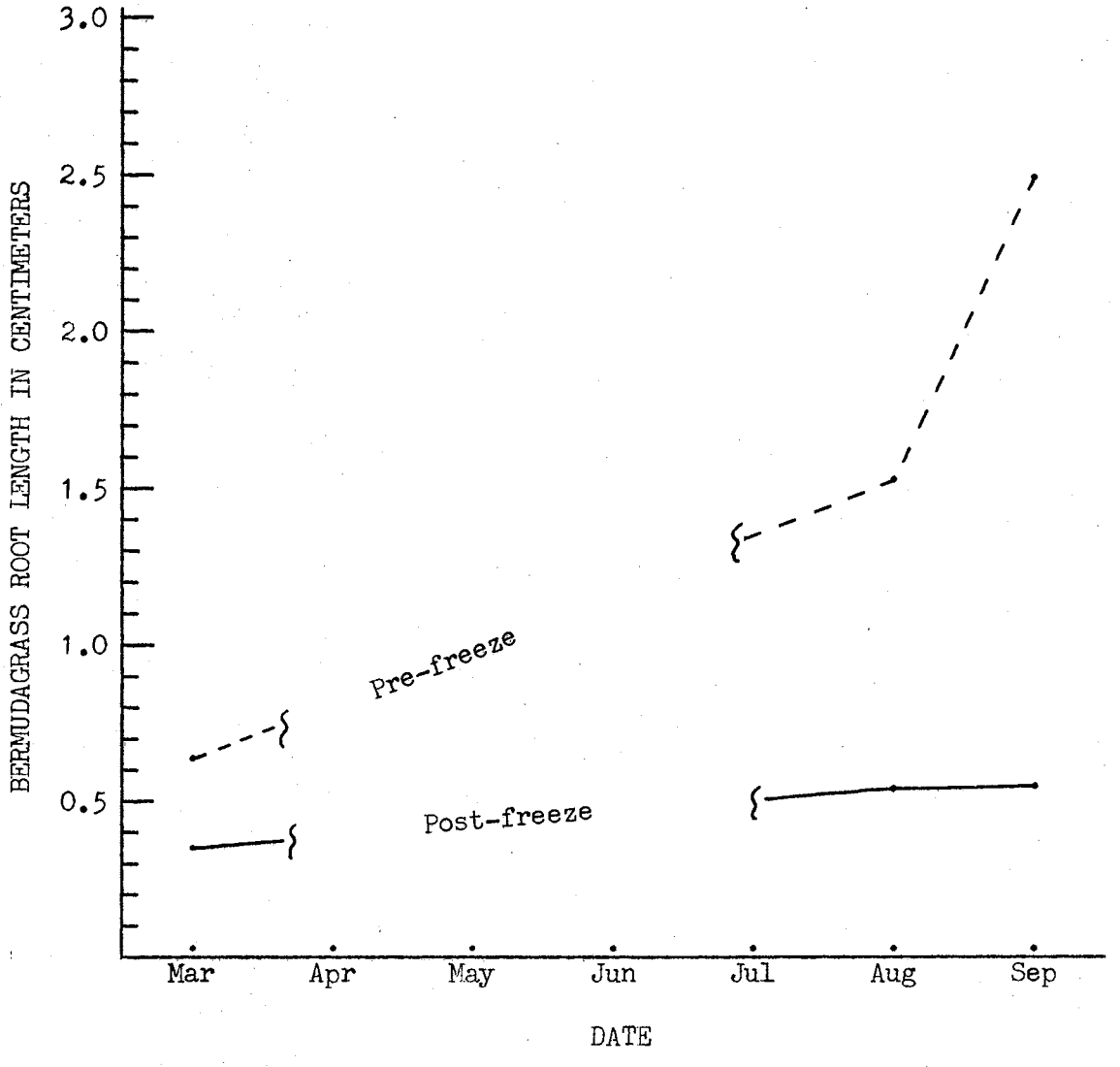

Figure 9. Length of Roots in Bermudagrass Sprigs Harvested in Three Months, Pre- and Post-Freeze

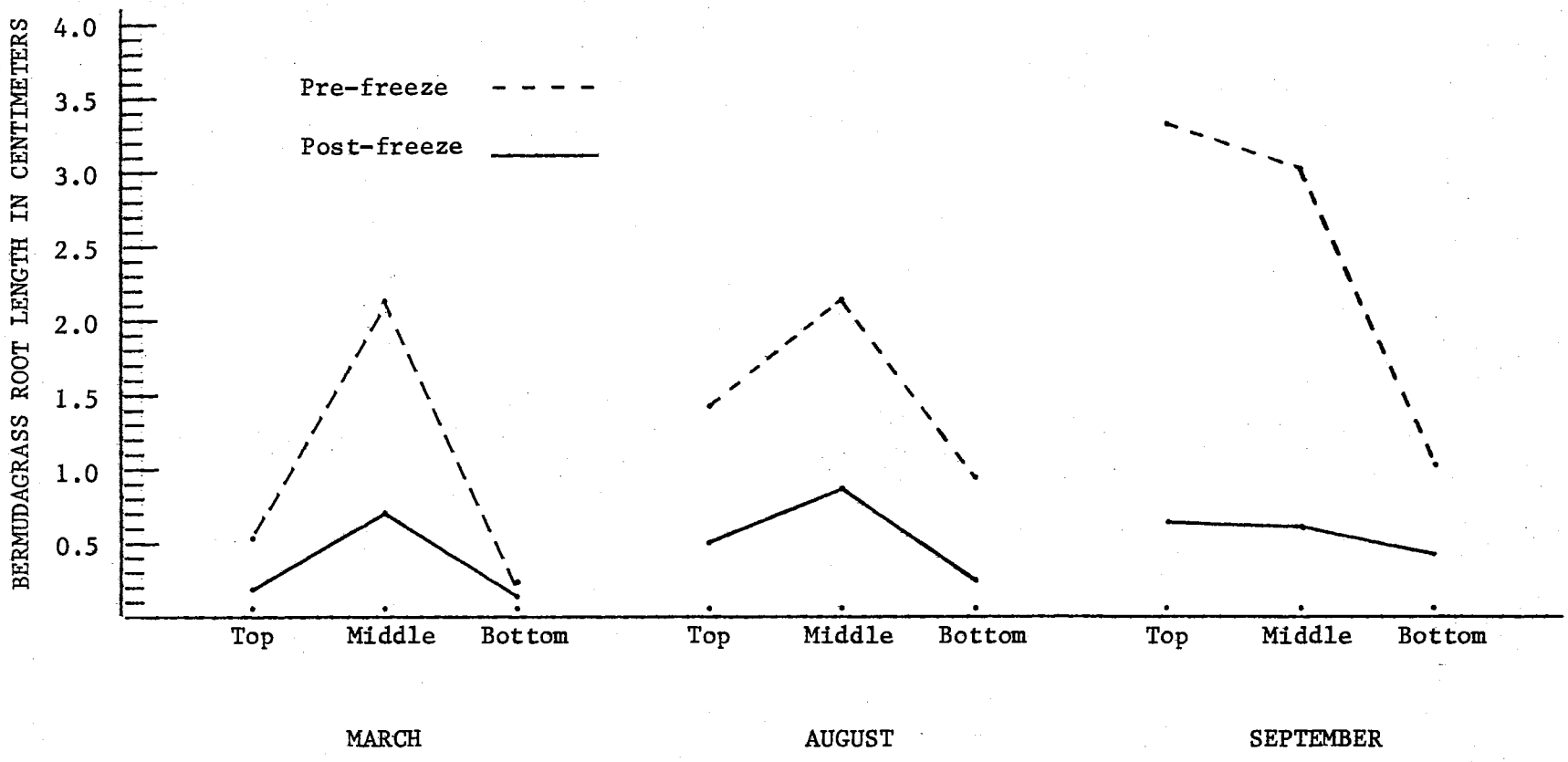

Figure 10. Length of Roots at Three Nodes, Top, Middle and Bottom, in Bermudagrass Sprigs Harvested in Three Different Months, Pre- and Post-Freeze

the pre-freeze and post-freeze treatments. However, the September sprigs had the longest roots at the top node and the roots at the middle and bottom nodes were successively shorter for both freezing treatments. Both the month and the freeze influenced the root length at the various nodes as did the combination of the month and freeze together. All of these factors produced differences in root length which were significant at the 1% level. The analysis of variance for these factors is presented in the Appendix, Table XI, page 103.

The previous explanation of the undamaged areas above and below the mid-node could explain the data for March and August. The possibility that the upper node was damaged by being exposed to air and the bottom node damaged by excess moisture would also fit the data for these months. However, the changes in the September root lengths would suggest that possibly the additional moisture in the vermiculite might have caused the reduction in root length. Since it is unlikely that the moisture would influence the September sprigs in such a manner and not also affect the sprigs harvested in March and August, it is doubtful if this is the primary cause of the root length reductions. The same reasoning could apply to the undamaged mid-node vs the damaged end-node hypothesis. The apparent reasons for the differences in length, such as those above, can be rejected due to these various discrepancies. Consequently, no reasonable hypothesis can be provided at the present time which will adequately explain the data obtained.

Greenhouse Experiment

The results of the greenhouse experiment were unsatisfactory, due primarily to faulty technique used in the intended freezing test. The

growth chamber used in this test was a chest-type box, with interior dimensions of approximately 0.6-1.2 meters (two by four feet). Fans provided a continuous circulation of air inside the chamber. Prior to placing the potted plants in the chamber, a recording thermometer had been used to check the temperature differences at opposite ends of the chamber. It was found that the temperature was approximately 1.1° C warmer at one end of the chamber than the other. Since this temperature differential remained the same at any temperature checked, and since the experiment was arranged in a randomized block design which was blocked in the direction for the temperature change, it was felt that this amount of variation would not be overly deterimental.

When the 54 pots were placed in the chamber, they were touching at the top, and there was less than an inch of space between the pots and the side of the chamber as shown in Figure 11. When the temperature was lowered in the actual freezing test, it was found that with the plants in the chamber, the temperature was much more uneven. The 1.1° C difference increased at one point to over a 11.7° C difference. By using the average temperature of the 14 replications, a result was obtained which had some plants at one end of the chamber being frozen below -8.9° C for over three hours, and some at the other end never getting as low as -8.9° C. The temperatures for the individual pots were taken approximately five centimeters from the edge of the pots and are given in the Appendix, Table XII, page 105. It was found after the plants had been returned to the greenhouse, that all except four plants had been killed.

The earlier work on killing temperatures had been done on bare sprigs in a corked test tube. The assumption was made that rhizomes

Figure 11. Photograph of Bermudagrass Pots in the Growth Chamber

with a soil cover would react to temperatures similarly to the bare sprigs. Since an insufficient number of plants survived the freezing test, no valid evaluation on the effects of the various fertility ratios on coldhardiness of the plants could be made.

Laboratory Experiments

Electrolysis Study

There were only two factors in the electrolysis study which would seem to be significant and these were the potash level and the replications. Both were significant at the 1% level.

The percent electrolytes increased steadily from replication one to three. This would indicate that there was a difference between the replications which resulted in more cells being damaged in the third replication by the freeze treatment than in the second replication, and more in the second replication than in the first.

The same trend existed in the sprouting tests. The number of live sprigs decreased from the first replication to the third, although the decrease from the first replication to the second was not nearly as great as from the second to the third replication. The differences in electrolyte percentages and the number of live sprigs is shown in Figure 12. There is a definite slope across the field from the first to the third replication. Other than this, the only explanation that can be offered is the one previously given (see page 38).

The differences in the effect of potassium levels are also unexplainable from the results obtained. The results of the zero and 112 kg potassium treatments were approximately the same, while the 56 kg

per hectare potassium treatment showed a 17% reduction in cell damage. Three soil samples were taken from areas near the test, but not included in it. The potassium levels varied from 297-1177 kilograms per hectare. With this amount of variation present, it is obvious that little reliance can be placed on the fertility differences obtained in the field experiment.

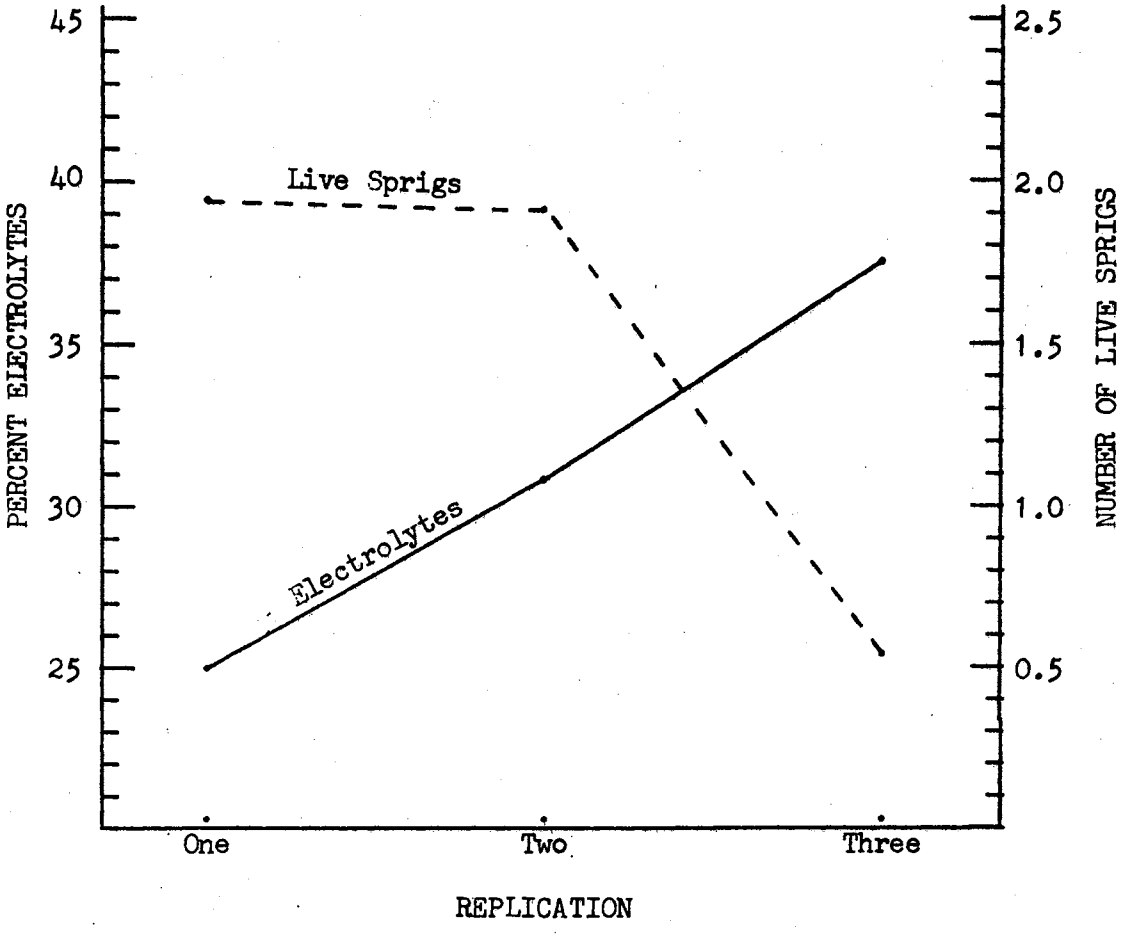

Figure 12. Percent of Electrolytes Present After Freezing Compared With the Number of Live Sprigs After Freezing in Bermudagrass Rhizomes Harvested From Three Replications*

*The numbers used in the live sprig determinations were the means of 378 sprigs in each replication.

Correlation coefficients were computed for the electrolysis readings and the number of live sprigs in the sprouting test, for the electrolysis readings and the percent sugar in the sprigs, and for the percent sugar and the number of live sprigs in the sprouting test.

The percent sugar and electrolysis readings, and the number of live sprigs and the electrolysis readings, were negatively correlated and significant at the 1% level, while the percent sugar and the number of live sprigs were positively correlated and significant at the 5% level. The computations for these correlations are given in the Appendix, Table XIII, page 106.

These data would indicate that the higher the electrolysis reading, the fewer live sprigs are to be expected. Since the high electrolysis reading indicates greater cell damage, this is what would be expected. The findings also indicate that the greater the sugar readings the lower the cell damage. This would substantiate the work of the others who have found that high sugar levels provide some amount of cold protection. The data also show that the higher the sugar level, the greater the number of live sprigs. This would also be expected if the sugar did indeed provide some measure of protection during freezing.

From these results, it would appear that the sprouting tests in future work could be abandoned and the electrolysis test used in its place. Since the sprouting tests took 21 days and the electrolysis tests required only two days, this would be a considerable saving in time as well as equipment and space. The problems of mold, mechanical failure, and other factors which would tend to bias the findings would also be greatly reduced.

Sugar Determinations of Greenhouse Experiment

An analysis of the sugar content of bermudagrass sprigs in the greenhouse fertility experiment showed that only two factors were significant in their effect, treatments and replications, both of which were significant at the 1% level.

The difference in replications could be attributed to environmental effects either within the greenhouse, or within the growth chamber, or both. The most probable influencing factor was temperature, since it was known to have varied within the growth chamber, and it could have varied in the greenhouse sufficiently to cause the replication differences.

The difference in sugar content of the rhizomes as influenced by different nitrogen, phosphorus and potassium ratios is shown in Figure 13. There was nearly a 25% decrease in sugar content from the treatment with the highest sugar level to the treatment with the lowest sugar level. According to Duncan's New Multiple Range Test (18), at the 5% level the 1-2-1, 4-0-0 and 4-1-6 treatments were not significantly different. The 4-1-3 and 4-1-6 treatments also showed no significant variation although the 4-1-3 treatment was significantly less than either the 4-0-0 or the 1-2-1 treatments.

If sugar was the primary factor influencing coldhardiness, this data would disagree with the findings of Gilbert and Davis (21) who worked with three of these same fertility ratios. The 4-0-0 ratio in their study had the least coldhardiness, while the 4-1-6 and 4-1-3 ratios had the most coldhardiness. According to their findings, the 4-0-0 treatment should have had the lowest sugar content and the 4-1-6

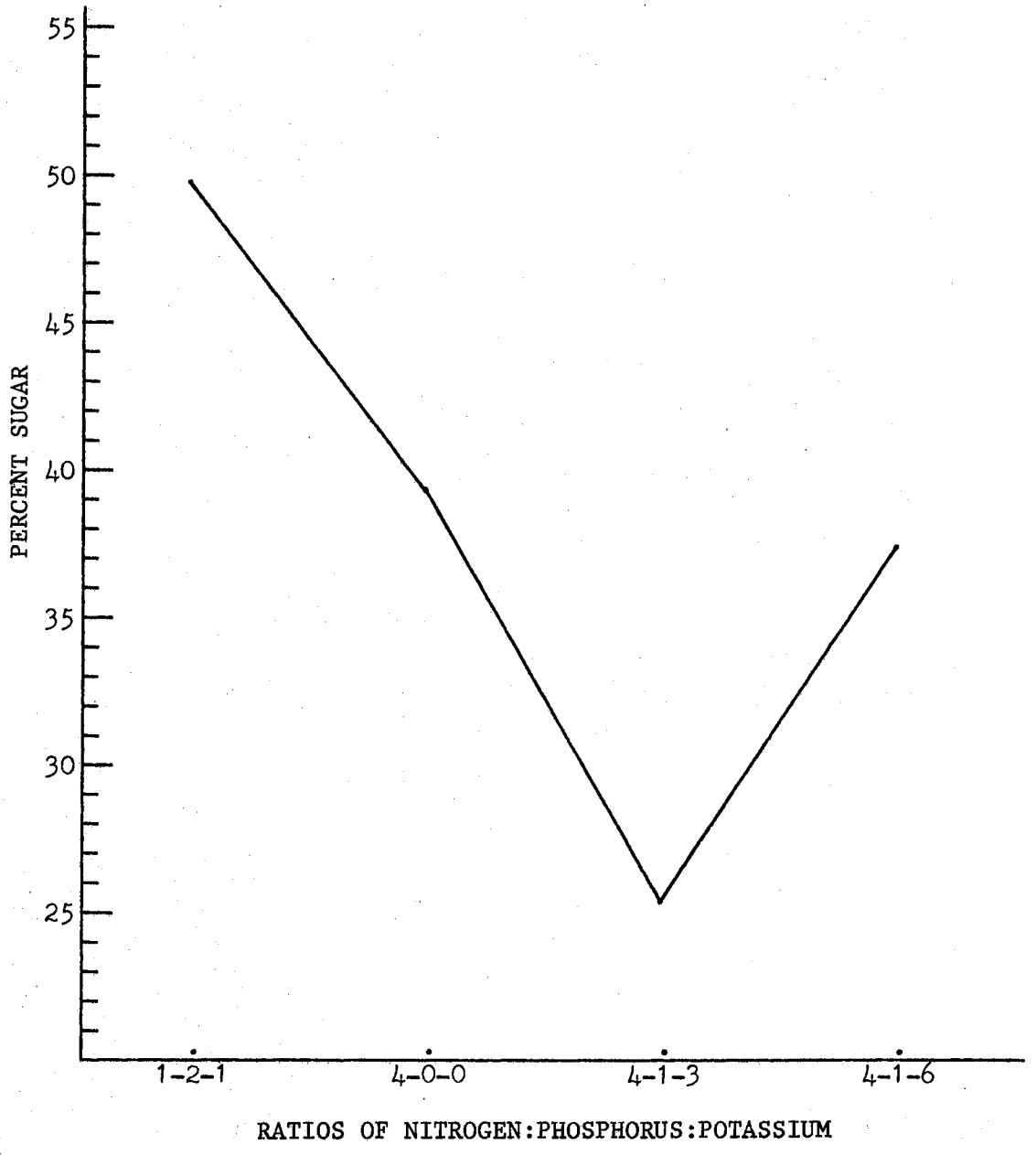

Figure 13. Average Percent Sugar in Bermudagrass Rhizomes As Determined by Two Anthrone Analyses for Four Fertility Ratios

and 4-1-3 ratios should have had high sugar contents with the 1-2-1 ratio being intermediate.

Since all of the plants in this investigation were killed, no conclusions can be made. Reeves and his group (77) however, might provide a partial explanation of the total kill. They found that the sugar content affected coldhardiness in bermudagrass, but only in a range of 1-2° C. Since the variations in most of the cold chamber exceeded this range considerably, a larger number of damaged plants would be expected.

Sugar Identification

A summarization of the sugars which were tentatively identified as being either absent or present, and the method used in the identification, is shown in Table VI. It is at once apparent that some sugars are listed as being both present and absent. The explanation for this is that the sugar was probably present in a very small amount, and the solvent system used for one determination was not sufficiently sensitive for them to be identified, while another, more sensitive system, did make the separation well enough for them to be identified.

Some of the sugars identified by the auto-analyzer were also not identified in the chromatography determinations. Most of these were present in very small amounts and the same explanation would apply, the chromatography systems used were not sufficiently sensitive to identify such small quantities.

On Plate 1 the unknown developed four spots with Rf values of 81, 48, 24, and 20. Raffinose and trehalose were eliminated since both had an Rf value of eight. Galactose was tentatively identified as being present with an Rf of 19. The Rf values of the remaining sugars were

TABLE VI

SUMMARIZATION OF SUGARS TENTATIVELY IDENTIFIED AS BEING PRESENT AND OTHER SUGARS CHROMATOGRAPHED BUT NOT FOUND IN COMMON BERMUDAGRASS RHIZOMES AND THE METHOD USED IN IDENTIFICATION

SUGARS PRESENT		SUGAR STANDARDS CHROMATOGRAPHED BUT NOT FOUND
Chromatography	Auto-Analyzer	
Galactose	Galactose	Galacturonic Acid
Rhamnose	Rhamnose	Arabinose
Sucrose	Glucose	Galactose
Mannose	Mannose	Raffinose
Maltose	Fucose	Trehalose
	Ribose	Fructose
	Xylose	Maltose
		Ribose
		Xylose

so close that identification was impossible. A reproduction of Plate 1 is shown in Figure 14.

On Plate 2 the unknown showed five spots with Rf values of 99, 55, 29, 20, and 11. The spot with the Rf of 55 was apparent only under ultraviolet (UV) light and was quite faint. It was tentatively identified as rhamnose which had an Rf of 53 and had a blue-green color with a yellow cast to it. Maltose with an Rf of 11 was also tentatively identified. Xylose with an Rf of 44 developed a yellow-green color and was eliminated, as was ribose with an Rf of 39. Ribose also developed a bright blue-green color. Arabinose was a pale blue and mannose was a pale green under UV light. Fructose, sucrose, glucose, and galactose were all a bright yellow and ran too close together for separation. Plate 2 is shown in Figure 15.

On Plate 3 the unknown developed three spots with Rf values of 35, 26, and 7. Galacturonic acid with an Rf of 47 was eliminated due both to its Rf value and its color. The spot developed a bright purple color which changed to an orange-violet under UV light. Maltose was also eliminated with an Rf of 17. Sucrose broke into two spots with Rf values of 36 and 26 and was tentatively identified. Plate 3 is shown in Figure 16.

On Plate 4 the unknown had three spots with Rf values of 49, 38, and 13. Arabinose with an Rf of 58 was eliminated. It also developed a bright blue color under UV light which was not present in the unknown. Maltose with an Rf of 31 was again eliminated. Sucrose again separated into two spots with Rf values of 50 and 42 and was again tentatively identified as being present. Figure 17 shows a reproduction of Plate 4.

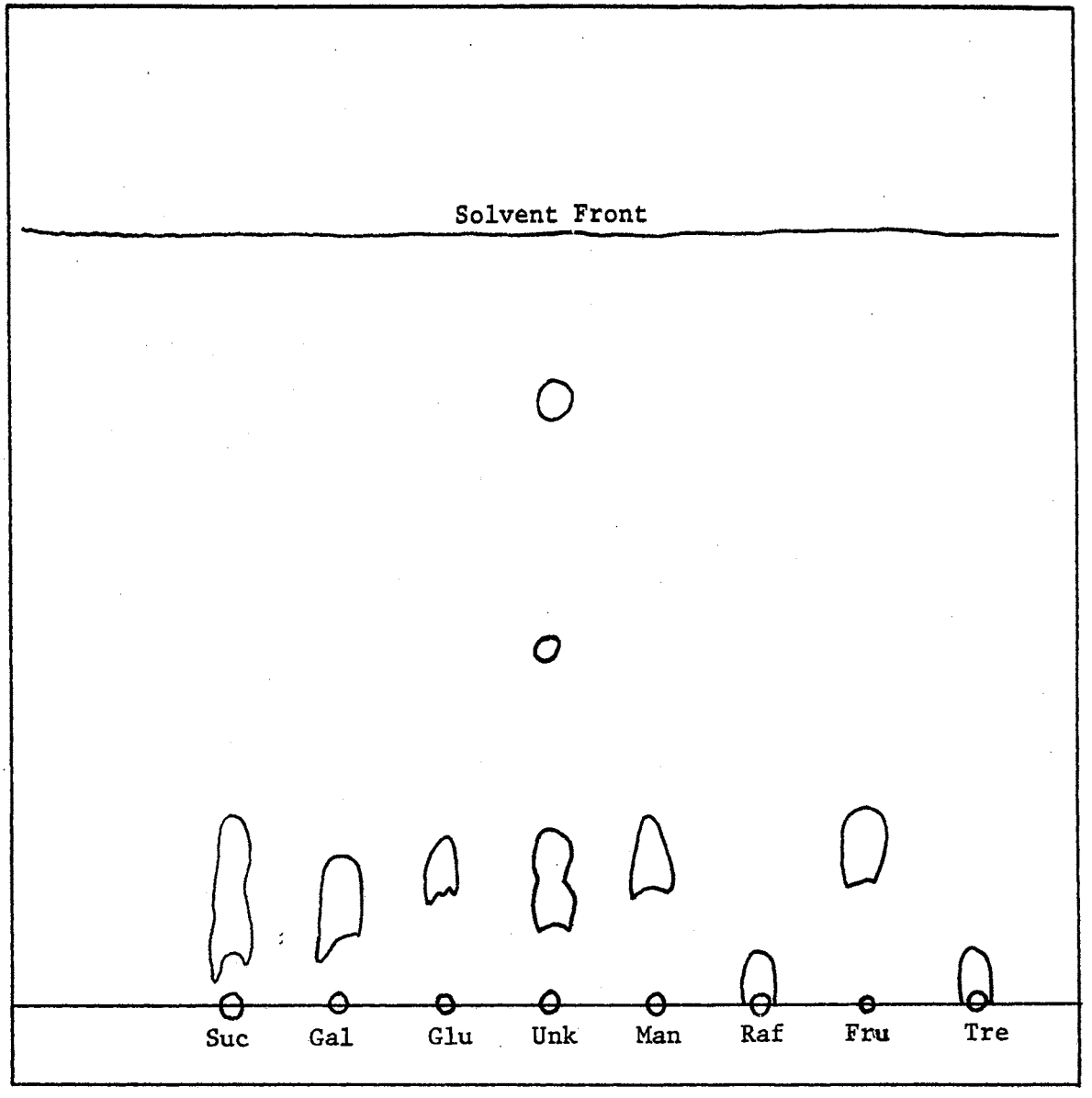

Figure 14. Reproduction of Chromatographic Separation of Sugars Obtained From Common Bermudagrass Rhizomes Compared With Seven Known Carbohydrates on Plate 1 Reduced to Three-Quarter Size

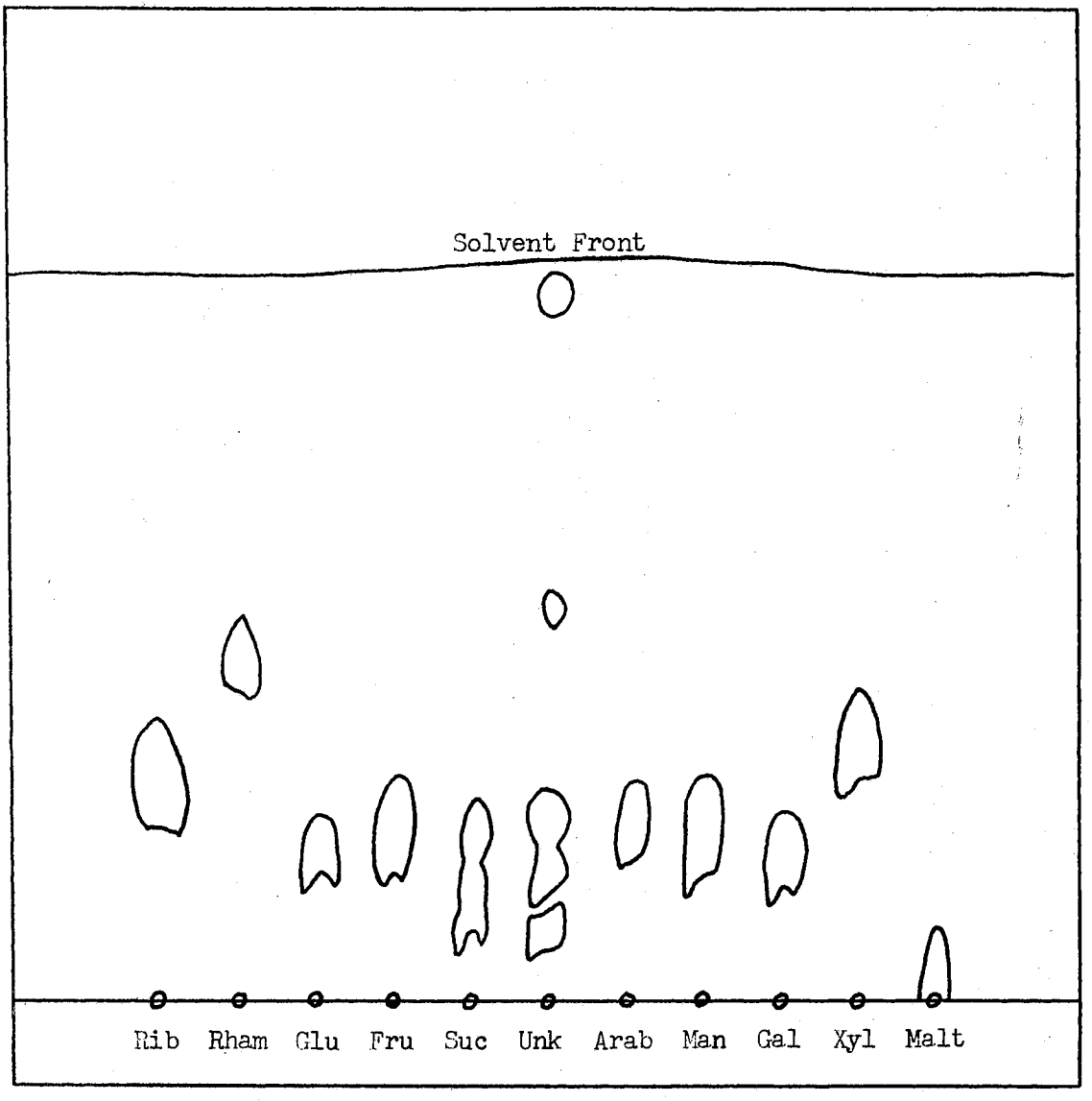

Figure 15. Reproduction of Chromatographic Separation of Sugars Obtained From Common Bermudagrass Rhizomes Compared With Ten Known Carbohydrates on Plate 2 Reduced to Three-Quarter Size

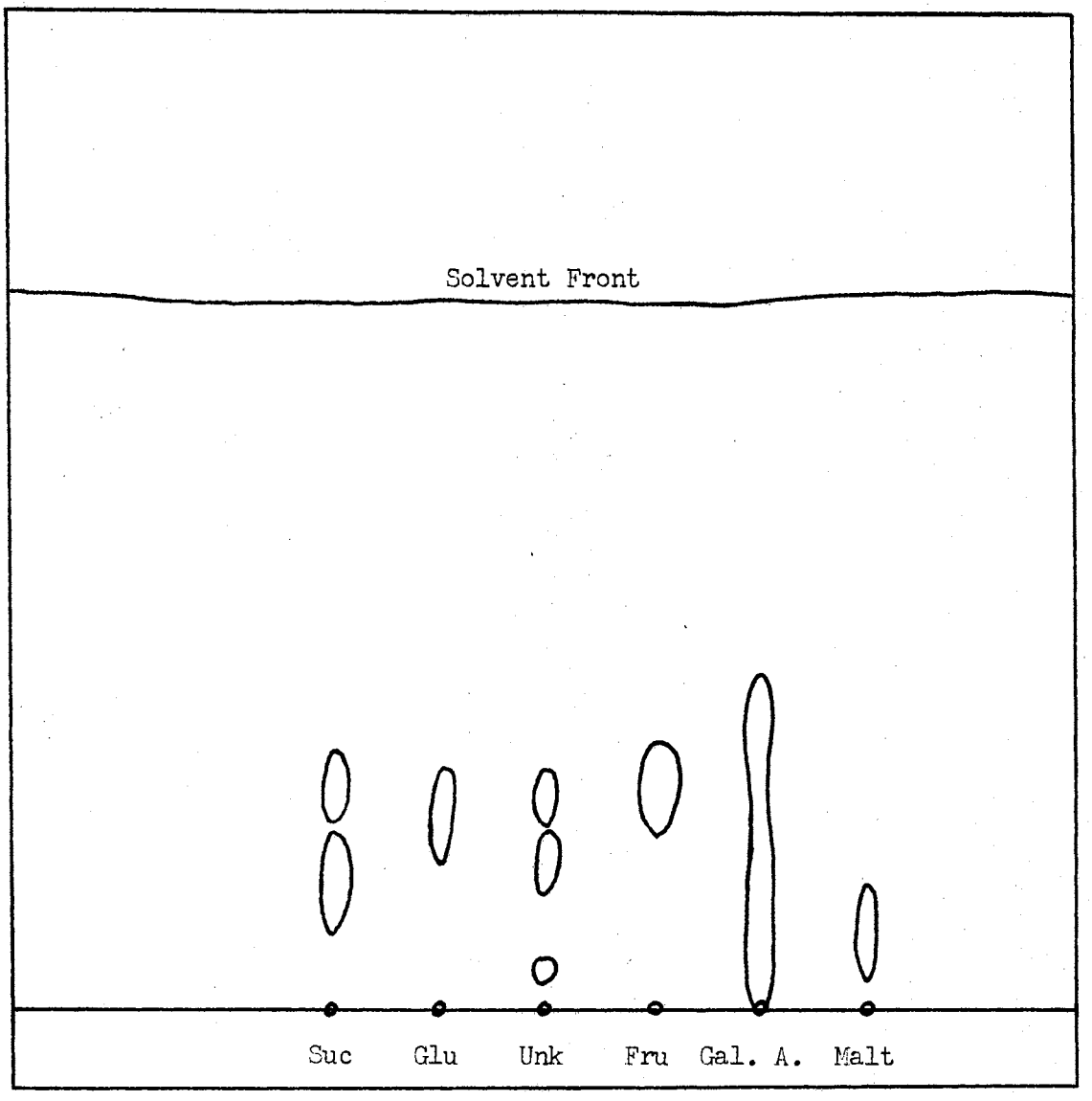

Figure 16. Reproduction of Chromatographic Separation of Sugars Obtained From Common Bermudagrass Rhizomes Compared With Five Known Carbohydrates on Plate 3 Reduced to Three-Quarter Size

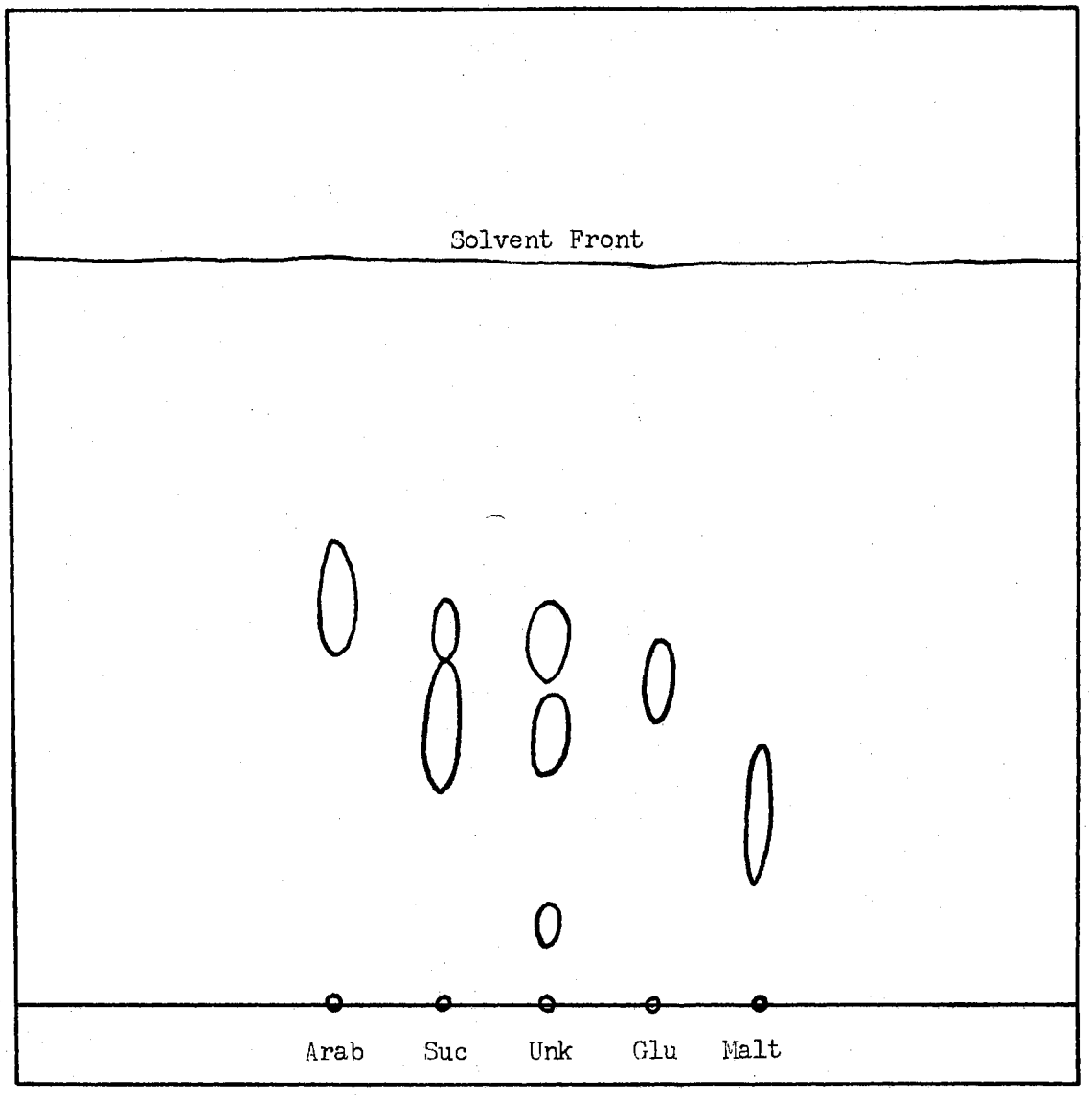

Figure 17. Reproduction of Chromatographic Separation of Sugars Obtained From Common Bermudagrass Rhizomes Compared With Four Known Carbohydrates on Plate 4 Reduced to Three-Quarter Size

On Plate 5, in the first solvent system, the unknown developed three spots with Rf values of 74, 62, and 30. Sucrose had an Rf of 74 and fructose was 75. In the second solvent, the Rf's of the unknown were 26, 10, and two. Fructose had an Rf of 34 and was eliminated while sucrose had an Rf of 27 and was tentatively identified as being present. Plate 5 is shown in Figure 18.

In solvent number five, on Plate 6, the unknown developed two spots with Rf values of 25 and three. The Rf values of sucrose, glucose and fructose were 24, 29, and 30 respectively. In solvent number three, the unknown Rf values were 38 and 16 while sucrose and fructose were both 39 and glucose was 45. Sucrose was once again tentatively identified while glucose and fructose were eliminated. Plate 6 is shown in Figure 19.

On the Whatman paper, the unknown developed two spots with Rf values of 31 and 13. Mannose was tentatively identified as being present with an Rf of 30 and galactose was eliminated with an Rf of 23. Glucose did not appear as being present at this concentration. Under UV light, the unknown spots both showed a bright orange color, while the galactose and mannose both showed a lighter yellow color. A reproduction of the Whatman paper strip is shown in Figure 20.

In an attempt to verify some of the above findings, a hydrolyzed and an unhydrolyzed sample of the unknown were run through a Technicon Auto-analyzer which had been calibrated to identify and show the relative amounts present of seven sugars. The sugars which could be identified were rhamnose, ribose, mannose, fucose, galactose, xylose and glucose. The chart of the two samples after being analyzed is shown in Figures 21 and 22. It is unfortunate that the Auto-analyzer was not

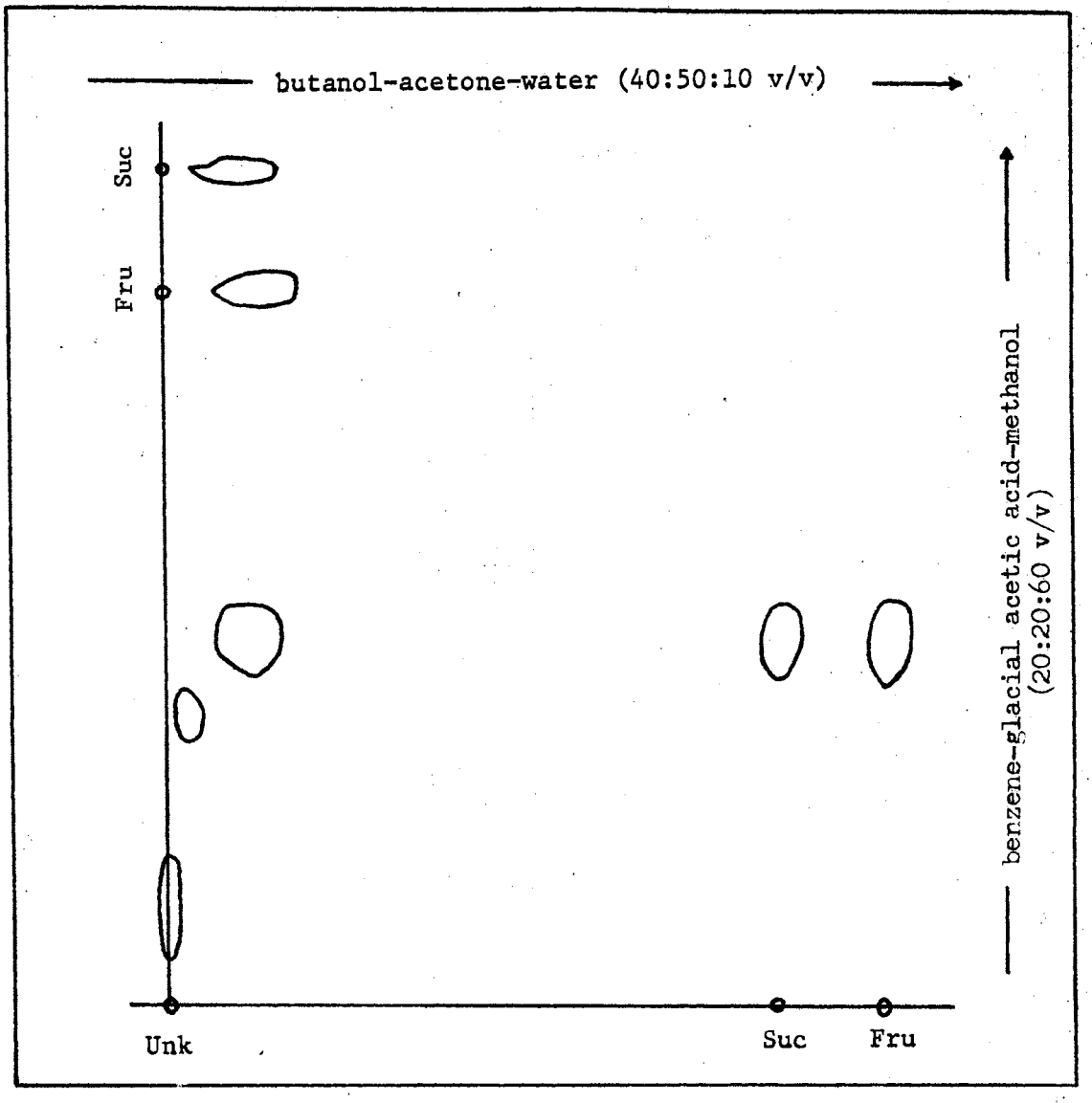

Figure 18. Reproduction of Chromatographic Separation of Sugars Obtained From Common Bermudagrass Rhizomes Compared With Two Known Carbohydrates on Plate 5 Reduced to Three-Quarter Size

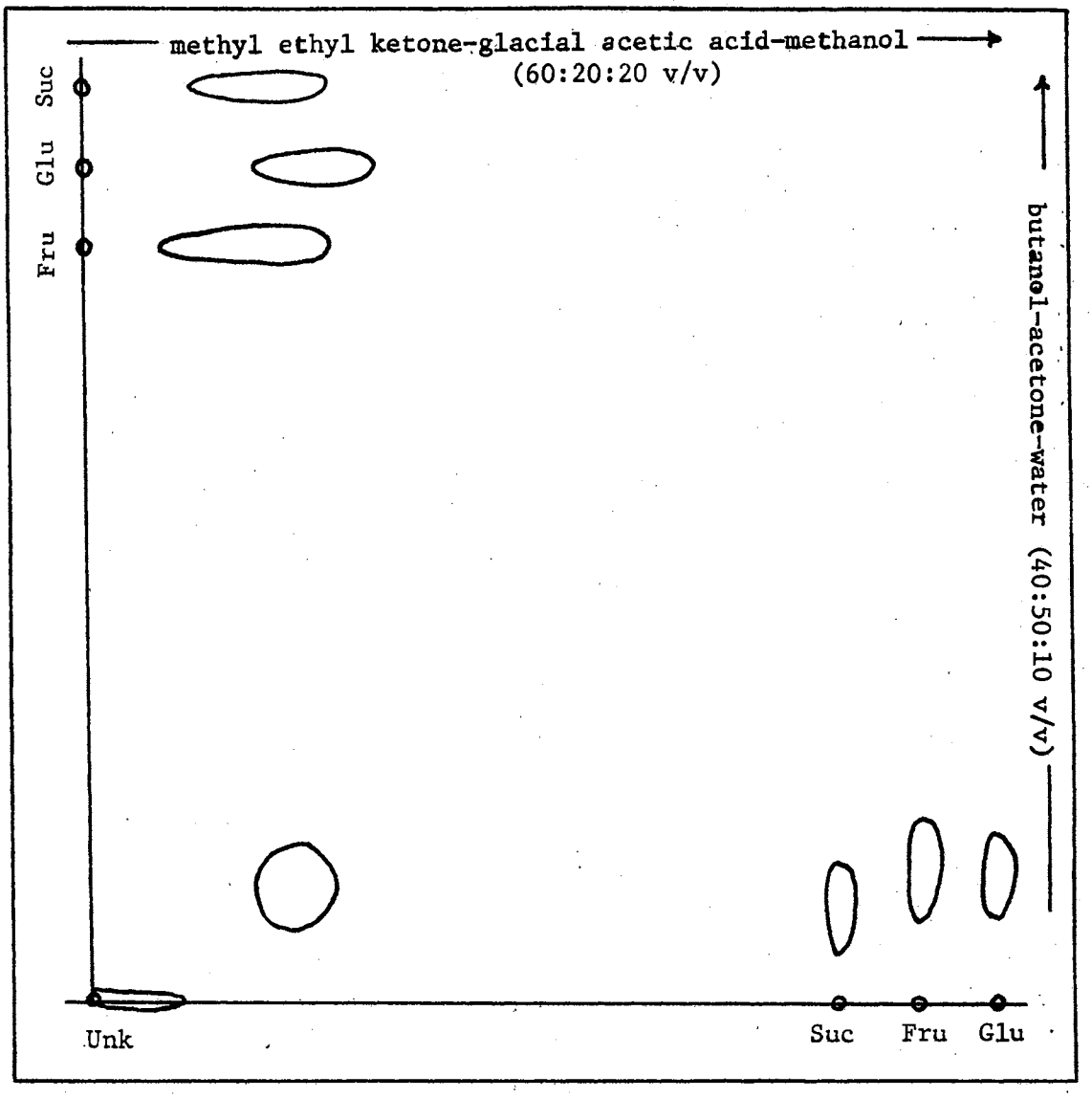

Figure 19. Reproduction of Chromatographic Separation of Sugars Obtained From Common Bermudagrass Rhizomes Compared With Three Known Carbohydrates on Plate 6 Reduced to Three-Quarter Size

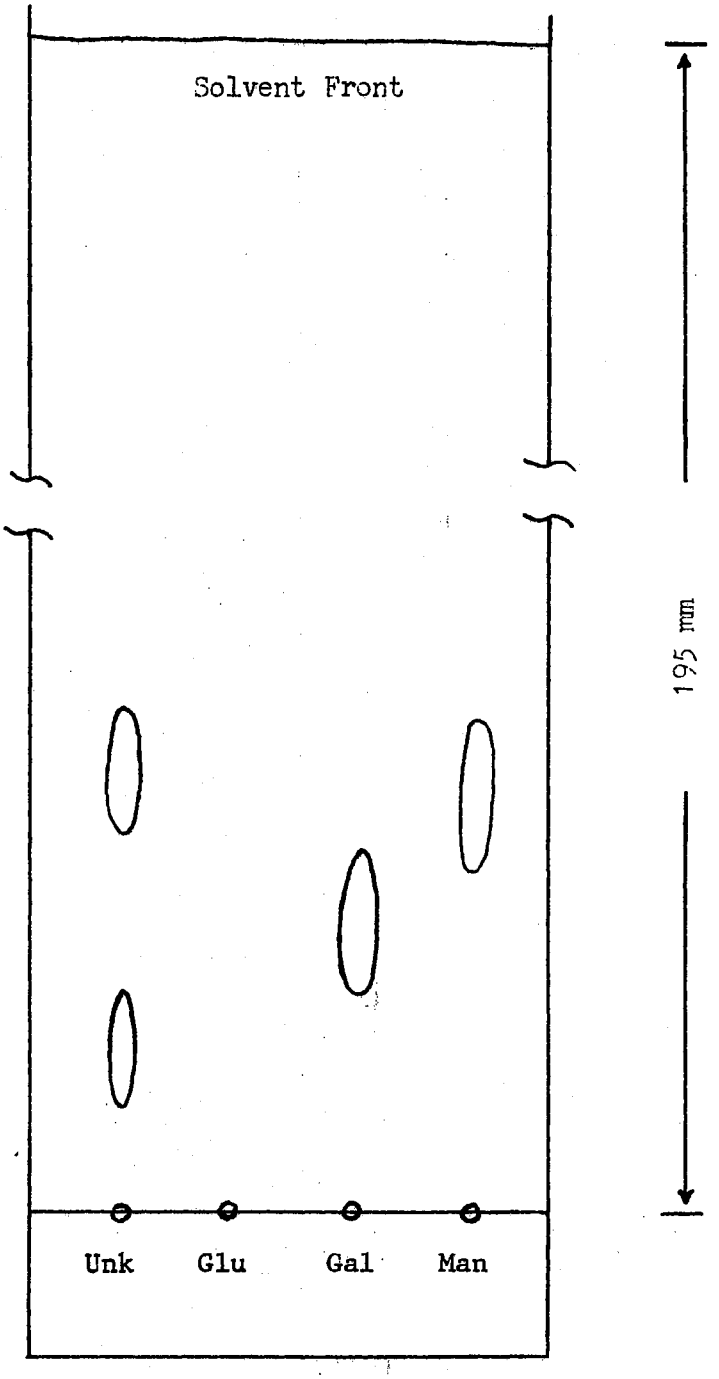

Figure 20. Reproduction of Chromatographic Separation of Sugars Obtained From Common Bermudagrass Rhizomes Compared With Three Known Carbohydrates on Whatman Paper Reduced to Three-Quarter Size

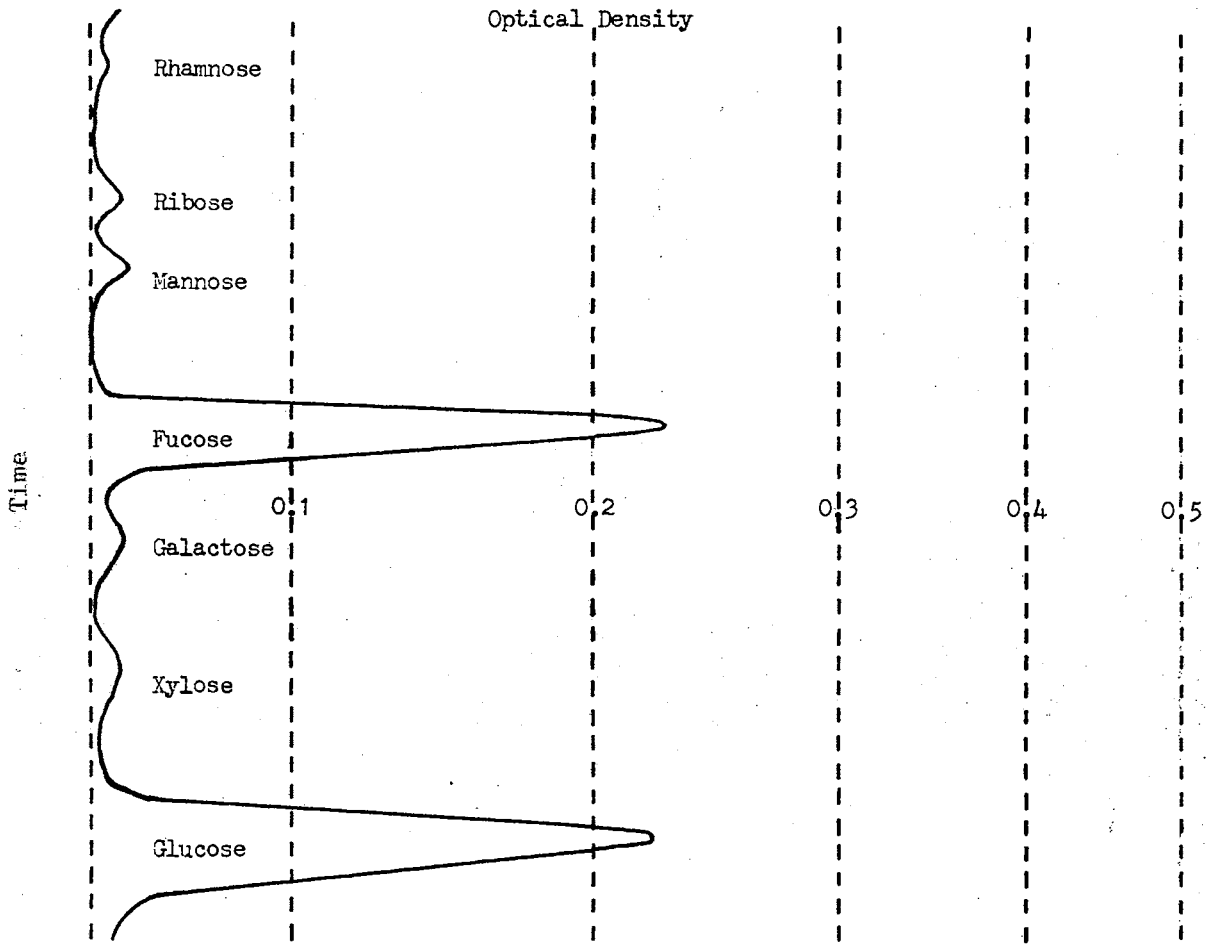

Figure 21. Technicon Auto-Analyzer Graph Showing Location and Amounts of Sugars in a Hydrolyzed Sample of an Alcohol Extract From Common Bermudagrass Rhizomes

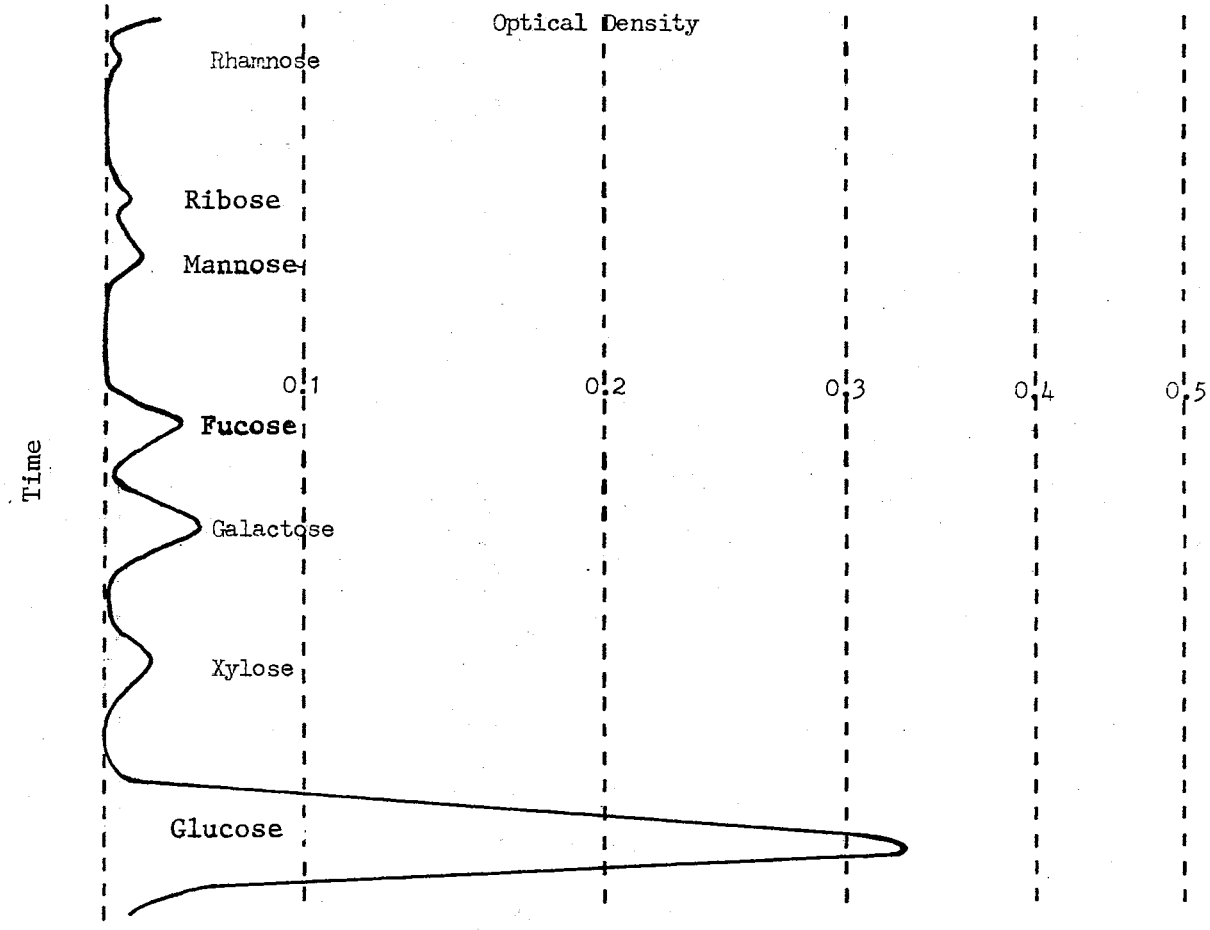

Figure 22. Technicon Auto-Analyzer Graph Showing Location and Relative Amounts of Sugars in an Unhydrolyzed Sample of an Alcohol Extract From Common Bermudagrass Rhizomes

calibrated to identify the other commonly occurring plant sugars. There could have been several other sugars present in the bermudagrass rhizomes which were not identified by either of the methods used.

In the hydrolyzed sample, rhamnose, ribose, mannose and xylose were present but made up less than nine percent of the total sugars, with rhamnose being less than one percent. Fucose and galactose were approximately five and a half and nine percent respectively of the total sample. Glucose made up the major portion of the sugars identified, comprising nearly 79% of the total.

In the unhydrolyzed sample, the same results were obtained for rhamnose and mannose. However, galactose decreased to about 3.6% and xylose decreased to 2.3% of the total. The biggest changes occurred in fucose and glucose. It was known that some fucose was destroyed during hydrolysis and an increase was expected. Yet the size of the increase, from 5.4% to 41.6%, was not expected. The amount of glucose also decreased from nearly 79% to about 47%. Since sucrose had been identified as being present in the sugar solution, it was expected that the hydrolyzed sample would have considerably more glucose than the unhydrolyzed sample.

The small amounts of xylose and ribose present in the unknown as disclosed by the auto-analyzer samples would explain why they would not be identified in the chromatography tests. The amounts present were too small to be detected in the system used. The systems were more selective for rhamnose and galactose, and the small amounts of the sugars present were shown.

Although both sucrose and glucose were identified, fructose was not. Since fructose was obviously present in the plant, three

explanations could account for the failure to identify it in the unknown sugar sample. First, fructose could exist in the plant only momentarily before it combines with glucose to form sucrose, and no free fructose exists in the plant. Secondly, the free fructose is present in very small amounts due to its rapid combination with glucose, and these minute amounts were not detectable in the systems used. Finally, a solvent system was not found which would separate fructose from the other sugars present.

Since Adegbola and McKell (2) analyzed Coastal bermudagrass for glucose, fructose and sucrose and found all three, it is likely that the last suggestion is more probable than the other two. However, DeCugnac (70), who determined that the principal storage form of carbohydrate in semi-tropical grasses was sucrose and starch, also found that these grasses did not store fructosans. Consequently, the possibility of the second suggestion occurring cannot be completely eliminated without further information.

The following solvent systems were used with both active and inactive plates but inadequate separation was obtained:

1. Formic acid-methyl ethyl ketone-tertiary butanol-water (15:30:40:15 v/v) (106)
2. 65 ml of ethyl acetate and 35 ml of isoproponol-water (2:1 v/v)
3. Isoproponol-pyridine-glacial acetic acid-water (80:80:10:40 v/v)
4. One normal butanol-glacial acetic acid-water (60:30:10 v/v).

The following reagents were used with varying degrees of effectiveness:

1. See page 34.
2. See page 34.
3. p-anisidine phosphate as developed by Mukherjee and Srivastava (66). Only the filtrate was used.
4. A 0.1 Molar solution of p-anisidine and phthalic acid in 96% ethanol as used by Schweiger (89).
5. Sodium periodate as used by Clark (11).

Reagent number one gave consistently the best results except when boric acid impregnated plates were used. As reported by Stahl and Kaltenbach (99), this method of detection will not work on boric acid impregnated layers. The only time the resorcinol reagent (number two) was used, the results were so unsatisfactory that another reagent (number one) had to be used in conjunction with it.

CHAPTER V

SUMMARY AND CONCLUSIONS

A field investigation of the effect of 27 fertility treatments on the sugar content of common bermudagrass rhizomes was conducted in hopes of developing a rapid method of predicting quality and in turn cold-hardiness of propagating material of this grass. A simple method of determining sugar content of the rhizomes was found and used. In this investigation, the fertility treatments had no effect on the sugar content of the rhizomes. Based on these experiments it was concluded, after the investigation was completed, that the soil contained too much phosphorus and potassium from previous treatments to be useful in determining the effect of phosphorus and potassium treatments on carbohydrate production.

A coldhardiness experiment was conducted to determine a temperature which would kill 50% of the untreated rhizomes. After several preliminary trials, a temperature of -8.9° C for a two-hour period was used. After the test was completed (with over a 90% kill of the plants), it was concluded that some changes in technique were required. Due to the inability to prevent uneven temperatures within the growth chamber, and due to the difference in the way growing plants reacted compared to bare sprigs, it was concluded that plant material being tested should have the temperature lowered to a desired point. When this temperature has been reached, the plant should immediately be removed from the freezing chamber and placed in another chamber with a temperature of 1.1-1.7° C

to thaw. The interval when the plant remains in the freezing chamber for a definite time period, at a definite temperature, should be eliminated. Since the killing process is a function of both time and temperature, new killing temperatures would have to be determined. These would undoubtedly be considerably lower than the temperatures used in this experiment.

Sprouting and electrolysis studies were conducted to compare a 21-day sprouting test in a germinator with an electrolysis reading to see if results from the two tests were correlated. The latter test required only two days to complete. The technique of inserting the rhizomes vertically in moist vermiculite which was used in the sprouting test was discovered to be faulty. The top and bottom nodes of the sprigs were prevented from sprouting in some unknown manner while generally the middle node was favored in the formation of both leaves and roots. The results of the electrolysis investigations were closely correlated with those of the sprouting test. It is suggested that the electrolysis test be used where possible in lieu of the sprouting test.

The potassium levels caused significant differences in the electrolysis readings. With no potassium, or when 112 kg per hectare were added, considerable cell damage resulted. The 56 kg per hectare potassium level resulted in a significant reduction in cell damage. However, the tremendous differences in potassium levels in several areas outside of the plot area but nearby cast doubt on any of the fertility effects in the field experiment.

The electrolysis readings indicated that the higher the sugar levels in the rhizomes, the lower the amount of cell damage resulting from low temperatures. This is in agreement with other authors who have

worked in the field.

During the sprouting tests, the number of leaves and roots present, their location on the sprig, and the length of the roots was recorded. There was a significant number of leaves present at nodes which did not have roots. An application of 112 kg per hectare of phosphorus resulted in a significant reduction of roots after freezing, while the 56 kg rate increased the number of roots. However, the best result, a root at every node with a leaf, was obtained with no supplemental phosphorus fertilization. The number of nodes which had leaves without roots was also significantly lower in September than in August. The differences in the number of leaves and roots present on the rhizomes was totally unexpected and as yet no satisfactory explanation can be given for this phenomenon. It is recommended that further work be done in this area. Specifically, the influence of various amounts of rooting hormones on bermudagrass rhizomes should be determined. Fertility treatments, date and freeze treatment all influenced the numbers of leaves and roots present. The location of the node in relation to the vertical placement of the sprig in the sprouting tray also influenced the formation of leaves and roots. The importance of this whole problem became obvious when it was found that 93% of the live sprigs which initiated leaf growth failed to produce roots.

Root length was also influenced by the date of sprig harvest, the roots being longer on the September harvested sprigs than on those harvested in any of the other months tested. This was particularly true of the sprigs which were sprouted prior to freezing. After the sprigs had been frozen at -8.9° C for two hours, there was only a slight increase in the length of roots from March to September.

A greenhouse experiment using plants grown in a sterile sand was conducted using nutrient solutions with various ratios of nitrogen, phosphorus and potassium to determine their effect on sugar content and coldhardiness. The ratios had a significant effect on sugar content. However, since nearly all of the plants were killed during the freeze test, few conclusions can be made. The highest sugar levels were approximately 50, 40, and 37% and were found in the 1:2:1, 4:0:0, and 4:1:6 ratios respectively. There were no significant differences between these three treatments. The lowest sugar level, approximately 25%, was found in the 4:1:3 fertility treatment.

The highest sugar levels were not in agreement with the fertility ratios (4:1:3 and 4:1:6) shown to be the most coldhardy by other authors (21). It is recommended that this experiment be repeated with the previously suggested changes in the freezing procedure. If the highest sugar levels do not prove to be the most coldhardy, this would support the work of Reeves, McBee and Bloodworth (77) who found that the carbohydrate concentration in bermudagrass tissues only provided cold protection in a range of 1-2° C. Further work could then be directed toward identifying which factor present in bermudagrass actually does provide the majority of the cold protection.

An investigation was conducted to determine what sugars were present in bermudagrass rhizomes. Galactose, mannose, maltose, rhamnose and sucrose were tentatively identified through thin layer or paper chromatography. Fucose, galactose, glucose, mannose, ribose, rhamnose, and xylose were tentatively identified through use of a Technicon auto-analyzer. The auto-analyzer also gave relative amounts of the sugars present in the samples. Fucose and glucose made up nearly 90% of the

sugars present which the analyzer was calibrated to identify.

Although fructose was not identified individually, it also had to be present since a compound of which it is a constituent, sucrose, was found to be present.

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APPENDIX

TABLE VII

ANALYSIS OF VARIANCE OF SUGAR READINGS IN COMMON BERMUDAGRASS SPRIGS AS INFLUENCED BY FERTILIZER TREATMENTS IN THE FIELD

Source	df	SS	MS	F
Rep	2	48.76	24.38	5.21**
Nitrogen	2	0.66	0.33	
Phosphorus	2	14.00	7.00	
Nit x Phos	4	12.49	3.12	
Potassium	2	10.29	5.14	
Nit x Pot	4	26.59	6.65	
Phos x Pot	4	10.57	2.64	
Nit x Phos x Pot	8	41.61	5.20	
Error A 1/	52	243.17	4.68	
Month	6	4213.83	702.31	213.45**
Mo x Nit	12	34.79	2.90	
Mo x Phos	12	33.58	2.80	
Mo x Pot	12	46.44	3.87	
Mo x Rep	12	271.38	22.61	6.97**
Mo x Nit x Phos	24	93.16	3.88	
Mo x Nit x Pot	24	99.05	4.13	
Mo x Phos x Pot	24	81.75	3.41	
Mo x N x P x K	48	159.26	3.32	
Error B 2/	312	1026.54	3.29	

1/ Error A was obtained by combining the interactions which contained Rep without Month.

2/ Error B was obtained by combining all interactions containing both Rep and Month and at least one of the other factors in the factorial experiment,

** Significant at the .01 level.

TABLE VIII

ANALYSIS OF VARIANCE FOR THE NUMBER OF LIVE SPRIGS FOUND AFTER SPROUTING COMMON BERMUDAGRASS RHIZOMES AS INFLUENCED BY FERTILITY TREATMENTS IN THE FIELD AND A GROWTH CHAMBER FREEZING TEST

Source	df	SS	MS	F
Nitrogen	2	29.98	14.99	
Phosphorus	2	3.57	1.78	
Nit x Phos	4	81.54	20.39	
Potassium	2	33.12	16.56	
Nit x Pot	4	32.65	8.16	
Phos x Pot	4	20.84	5.21	
Month	2	1172.46	586.23	62.31**
Nit x Mo	4	0.54	0.14	
Phos x Mo	4	5.40	1.35	
Pot x Mo	4	7.28	1.82	
Error A 1/	48	451.59	9.41	
Freeze	1	2512.62	2512.62	480.85**
Nit x Freeze	2	6.57	3.28	
Phos x Freeze	2	1.86	0.93	
Pot x Freeze	2	4.46	2.23	
Mo x Freeze	2	617.27	308.64	59.07**
Error B 2/	72	376.22	5.23	

1/ Error A was obtained by combining all three factor, and higher, interactions which did not contain Freeze.

2/ Error B was obtained by combining all three factor, and higher, interactions which contained Freeze.

** Significant at the .01 level

TABLE IX

ANALYSIS OF VARIANCE FOR THE NUMBER OF LEAVES WITHOUT ROOTS FOUND AFTER SPROUTING COMMON BERMUDAGRASS SPRIGS AS INFLUENCED BY FERTILITY TREATMENTS IN THE FIELD AND A GROWTH CHAMBER FREEZING TEST

Source	df	SS	MS	F
Nitrogen	2	0.78	0.39	
Phosphorus	2	3.11	1.56	
Nit x Phos	4	9.11	2.28	
Potassium	2	0.70	0.35	
Nit x Pot	4	13.19	3.30	
Phos x Pot	4	34.07	8.52	
Month	2	51.81	25.91	6.26**
Nit x Mo	4	11.96	2.99	
Phos x Mo	4	14.52	3.63	
Pot x Mo	4	11.48	2.87	
Error A 1/	48	198.70	4.14	
Freeze	1	70.67	70.67	32.93**
Nit x Freeze	2	0.75	0.38	
Phos x Freeze	2	0.20	0.10	
Pot x Freeze	2	2.16	1.08	
Mo x Freeze	2	31.20	15.60	7.27**
Error B 2/	72	154.52	2.15	

1/ Error A was obtained by combining all three factor, and higher, interactions which did not contain Freeze.

2/ Error B was obtained by combining all three factor, and higher, interactions which contained Freeze.

** Significant at the .01 level

TABLE X

ANALYSIS OF VARIANCE FOR THE NUMBER OF ROOTS WITHOUT LEAVES FOUND AFTER SPROUTING COMMON BERMUDAGRASS SPRIGS AS INFLUENCED BY FERTILITY TREATMENTS IN THE FIELD AND A GROWTH CHAMBER FREEZING TEST

Source	df	SS	MS	F
Nitrogen	2	0.48	0.24	
Phosphorus	2	0.48	0.24	
Nit x Phos	4	0.70	0.18	
Potassium	2	0.11	0.06	
Nit x Pot	4	0.41	0.10	
Phos x Pot	4	0.30	0.07	
Month	2	1.81	0.91	5.16**
Nit x Mo	4	1.04	0.26	
Phos x Mo	4	1.48	0.37	
Pot x Mo	4	0.74	0.19	
Error A 1/	48	8.44	0.18	
Freeze	1	0.06	0.06	
Nit x Freeze	2	0.11	0.06	
Phos x Freeze	2	1.37	0.69	3.96*
Pot x Freeze	2	0.26	0.13	
Mo x Freeze	2	0.26	0.13	
Error B 2/	72	12.44	0.17	

1/ Error A was obtained by combining all three factor, and higher, interactions which did not contain Freeze.

2/ Error B was obtained by combining all three factor, and higher, interactions which contained Freeze.

** Significant at the .01 level

* Significant at the .05 level

TABLE XI

ANALYSIS OF VARIANCE FOR ROOT LENGTH FOUND AFTER SPROUTING COMMON BERMUDAGRASS SPRIGS AS INFLUENCED BY FERTILITY TREATMENTS IN THE FIELD AND A GROWTH CHAMBER FREEZING TEST

Source	df	SS	MS	F
Rep	2	102.18	51.09	
Nitrogen	2	3.25	1.63	
Phosphorus	2	8.07	4.04	
Nit x Phos	4	7.59	1.90	
Potassium	2	11.34	5.67	3.66*
Nit x Pot	4	2.81	0.70	
Phos x Pot	4	1.59	0.40	
Nit x Phos x Pot	8	13.42	1.68	
Error A 1/	52	80.69	1.55	
Month	2	260.24	130.12	57.59**
Mo x Nit	4	10.61	2.65	
Mo x Phos	4	4.88	1.22	
Mo x Pot	4	2.78	0.70	
Mo x Nit x Phos	8	38.66	4.83	
Mo x Phos x Pot	8	11.63	1.45	
Mo x Nit x Pot	8	14.25	1.78	
Mo x N x P x K	16	20.60	1.29	
Error B 2/	108	244.01	2.26	
Freeze	1	408.92	408.92	224.20**
Fre x Nit	2	0.87	0.43	
Fre x Phos	2	3.30	1.65	
Fre x Nit x Phos	4	6.52	1.63	
Fre x Pot	2	0.57	0.29	
Fre x Nit x Pot	4	6.27	1.57	
Fre x Phos x Pot	4	2.18	0.54	
Fre x N x P x K	8	15.81	1.98	
Fre x Mo	2	169.35	84.68	46.43**
Fre x Mo x Nit	4	6.48	1.62	
Fre x Mo x Phos	4	5.15	1.29	
Fre x Mo x N x P	8	15.84	1.98	
Fre x Mo x Pot	4	1.96	0.49	
Fre x Mo x N x K	8	4.74	0.59	
Fre x Mo x P x K	8	3.42	0.43	
Fre x Mo x N x P x K	16	20.53	1.28	
Error C 3/	162	295.47	1.82	

TABLE XI (Continued)

Source	df	SS	MS	F
Node	2	209.99	104.99	104.45**
Nod x Nit	4	2.83	0.71	
Nod x Phos	4	4.69	1.17	
Nod x Nit x Phos	8	8.83	1.10	
Nod x Pot	4	3.22	0.81	
Nod x Nit x Pot	8	1.65	0.21	
Nod x Phos x Pot	8	11.87	1.48	
Nod x N x P x K	16	18.72	1.17	
Nod x Mo	4	54.81	13.70	13.63**
Nod x Mo x Nit	8	10.74	1.34	
Nod x Mo x Phos	8	16.78	2.10	
Nod x Mo x N x P	16	14.02	0.88	
Nod x Mo x Pot	8	5.91	0.74	
Nod x Mo x N x K	16	11.13	0.70	
Nod x Mo x P x K	16	17.11	1.07	
Nod x Mo x N x P x K	32	18.37	0.57	
Nod x Freeze	2	63.02	31.51	31.35**
Nod x Fre x Nit	4	0.11	0.03	
Nod x Fre x Phos	4	4.64	1.16	
Nod x Fre x N x P	8	5.66	0.71	
Nod x Fre x Pot	4	2.13	0.53	
Nod x Fre x N x K	8	5.93	0.74	
Nod x Fre x P x K	8	8.12	1.02	
Nod x Fre x N x P x K	16	5.34	0.33	
Nod x Fre x Mo	4	50.11	12.53	12.46**
Nod x Fre x M x N	8	12.42	1.55	
Nod x Fre x M x P	8	14.88	1.86	
No x F x M x N x P	16	10.27	0.64	
No x F x M x K	8	4.05	0.51	
No x F x M x N x K	16	12.87	0.80	
No x F x M x P x K	16	6.76	0.42	
No x F x M x N x P x K	32	15.46	0.48	
Error D 4/	648	651.40	1.01	

1/ Error A was determined by combining all factors which contained Rep but did not contain Month, Freeze, or Node.

2/ Error B was determined by combining all factors which contained both Rep and Month.

3/ Error C was determined by combining all factors which contained both Rep and Freeze.

4/ Error D was determined by combining all factors which contained both Rep and Node.

* Significant at the .05 level. ** Significant at the .01 level.

TABLE XII

AIR TEMPERATURE, IN DEGREES CENTIGRADE, AND SOIL TEMPERATURES TAKEN AT APPROXIMATELY 2.5 CENTIMETERS DEEP AND 5.0 CENTIMETERS FROM THE EDGE OF 15.2 CENTIMETER DIAMETER POTS CONTAINING COMMON BERMUDAGRASS DURING A FREEZING TEST IN A GROWTH CHAMBER

Time	Air Temp ° C	Replication 1	2	3	4	5	6	7	8	9	10	11	12	13	14	Avg
0745	1.1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
0815	0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
0900	-2.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1000	-4.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1100	-6.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1200	-8.9	0	0	0	0	-1.0	-2.5	-2.5	-2.0	-3.0	0	-2.0	-2.5	-3.0	-2.5	-1.5
1300	-8.9	-3.0	0	0	-2.0	-5.0	-8.0	-9.0	-7.0	-9.0	-2.0	-7.0	-8.0	-9.0	-8.0	-5.5
1400	-8.9	-4.0	-2.0	-2.0	-4.0	-7.0	-11.0	-14.0	-12.0	-13.0	-5.0	-12.0	-12.0	-14.0	-12.0	-8.9
1500	-8.9	-5.0	-4.0	-5.0	-6.5	-9.0	-10.5	-11.5	-10.5	-10.5	-9.0	-11.0	-11.0	-10.5	-10.5	-8.9
1600	-8.9	-6.0	-5.5	-6.0	-7.5	-9.0	-9.5	-10.0	-10.0	-10.0	-9.0	-10.0	-9.0	-10.0	-9.0	-8.7
1700	-6.7	-5.0	-6.0	-6.5	-7.5	-8.0	-8.5	-8.5	-8.0	-8.5	-8.0	-8.5	-7.5	-8.0	-7.0	-7.5
1800	-4.4	-4.0	-5.0	-5.5	-6.0	-6.5	-6.0	-5.5	-5.5	-6.0	-6.0	-6.0	-5.0	-5.5	-5.0	-5.5
1900	-2.2	-2.0	-3.0	-3.5	-4.0	-4.5	-4.0	-3.0	-3.0	-3.5	-4.0	-4.0	-2.5	-3.0	-2.5	-3.2
2000	0	-1.0	-1.0	-1.5	-2.0	-2.0	-2.0	-1.0	-1.0	-1.0	-2.0	-1.5	-0.5	-1.0	-0.5	-1.3
2100	1.7	3.0	2.5	2.5	2.0	1.5	1.5	1.5	1.0	1.5	1.5	1.5	2.0	2.0	2.0	1.8

TABLE XIII

SUM OF SQUARES, CROSS PRODUCTS AND CORRELATION COEFFICIENTS FOR THE ELECTROLYSIS READINGS AND THE PERCENT SUGAR IN THE SPRIGS, AND FOR THE PERCENT SUGAR AND THE NUMBER OF LIVE SPRIGS IN THE SPROUTING TESTS OF COMMON BERMUDAGRASS RHIZOMES AS INFLUENCED BY FERTILITY TREATMENTS IN THE FIELD AND A GROWTH CHAMBER FREEZING TEST

Sum of Squares, Cross Products and Correlation Coefficients From Error A in the Analysis of Variance 1/

Source	% Sugar in Sprigs	Electrolysis Reading	Live Sprigs
% Sugar in Sprigs	152.84 (1.0)	0.70 (0.083)	15.68 (0.118)
Electrolysis Reading		0.49 (1.0)	-0.99 (-0.131)
Live Sprigs			115.17 (1.0)

Sum of Squares, Cross Products and Correlation Coefficients From Error B in the Analysis of Variance 1/

Source	% Sugar in Sprigs	Electrolysis Reading	Live Sprigs
% Sugar in Sprigs	173.79 (1.0)	-9.13 (-0.530)**	69.07 (0.352)*
Electrolysis Reading		1.77 (1.0)	-11.82 (-0.597)**
Live Sprigs			221.00 (1.0)

1/ The numbers in parenthesis are the correlation between the two variables where

$$r_{ij} = \frac{SS_{ij}}{\sqrt{(SS_{ii})\ (SS_{jj})}}.$$

* Significant at the .05 level

** Significant at the .01 level

VITA

William Wade Fuller

Candidate for the Degree of

Doctor of Philosophy

Thesis: INFLUENCE OF FERTILITY LEVELS ON SUGAR CONTENT AND COLDHARDINESS IN COMMON BERMUDAGRASS (Cynodon dactylon (L.) Pers) RHIZOMES, AND IDENTIFICATION OF SOME SUGARS

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